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SOME PROPERTIES OF THE CURLY-TOP VIRUS[1]

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INTRODUCTION

According to some plant pathologists the virus diseases of plants are divided into two groups, the mosaic and the yellows diseases. The mosaic diseases under optimum conditions cause a mottling of the leaves, usually in the growing tissues of the plants. Some of the mosaic diseases are highly infectious, while others are not, and some have not been transmitted mechanically. Some of the mosaic viruses have been shown to be filterable, but others are not. Intracellular, cell inclusions, or x-bodies are associated with many diseases of this group. Mosaic viruses are transmitted mostly by sucking insects, usually aphids, rarely by chewing insects. Some single species of aphids are associated with the dissemination of many separate mosaic viruses, other species with the spread of but one mosaic virus. The peach aphid *(Myzus persicae)* is associated with the dissemination of fourteen separate virus diseases, and the potato aphid *(Macrosiphum solanifolii)* is associated with the spread of six plant viruses.[57] Some insects are mechanical vectors of mosaic viruses, as in the case of cucumber mosaic,[15] and retain the virus for only a short time. The aphid vector of spinach blight retains the infective power for a period of five days.[38] Some mosaic diseases are transmitted through the seeds.

Kunkel[36] lists 24 virus diseases of plants outside of the mosaic group, including well-known destructive yellows diseases in California, such as curly top of sugar beets; yellows of china aster, celery, and lettuce;

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strawberry yellows; and leafroll of potato. "In the yellows type of disease, chlorosis is general throughout the affected parts."[36] Most of the virus diseases in this group have not been transmitted mechanically, except by budding or grafting, and hence plant pathologists have given less attention to the filterability of these viruses. Cell inclusions or x and y-bodies have been reported in this group of diseases. In some of these diseases a specific relation is believed to exist between a particular virus and its insect vector. Some of the viruses of these diseases are carried by a single species of leafhopper or aphid. In the case of potato leafroll, however, the virus is transmitted by six species of aphids, two species of leafhoppers, a flea beetle *(Psylliodes affinis)*, and the larva of *Tipula paludosa*.[57] An incubation period occurs in some of the insect vectors, such as *Eutettix tenellus*,[7, 48, 53, 60] which transmits curly top to a large number of host plants;[20, 50, 51, 52, 54] *Cicadula divisa (= C. sexnotata* Aucct.) which carries yellows to a large number of ornamental flowering plants;[35, 37] *Cicadulina mbila*[59] which transmits streak disease to corn; and *Myzus persicae*[18, 57] which transmits leafroll to potato. It has been demonstrated, however, that *Eutettix tenellus* transmits curly top in short periods, on rare occasions, probably by contamination of mouth parts.[53] Many insect vectors retain the infective power for long periods, sometimes throughout the life of the insect, and this has been assumed as evidence of a multiplication of the virus in the body of the insect. No disease in this group has been reported as passing through the seeds.

Chemical investigations by Dunlap[17] and other scientists on the two classes of virus diseases seem to indicate that in the mosaics there is a higher nitrogen and lower carbohydrate content than in healthy leaves, while in the yellows group there is a lower nitrogen and higher carbohydrate content.

The filterability of the curly-top virus from both diseased sugar beets and infective beet leafhoppers has been demonstrated.[55]

The investigations reported in this paper were undertaken on some other properties of the virus to determine whether or not it has characteristics which might further differentiate the yellows group from the mosaics. By transmission experiments with previously noninfective beet leafhoppers a study was made of the virus in the juices from various parts of diseased beets, the effect of aging on the virus under aerobic and anaerobic conditions, cultivation of the virus outside of living plants, resistance to drying in plant tissues and infective beet leafhoppers, inactivation of virus with juices from an immune host plant, purification, dilution, thermal death point, and freezing of the virus.

GENERAL METHODS

Beet Extracts.—In the preparation of juice from the blades or petioles from diseased beets, the leaves were washed in distilled water, and ground to a pulp in a sterilized food chopper. In experiments reported in this paper the "beet root," that is, the sugar beet with the crown and lateral and tap roots removed, was used. The beet roots were scrubbed with a brush, sliced, and ground in a food chopper. The pulp was then placed in several layers of cheesecloth and the juice pressed out into a sterile pan by hand.

In earlier experiments[55] sterile beet-root juice was prepared by placing healthy beet roots, cut in small pieces, in an autoclave for a period of at least an hour, and the juice was extracted with steam pressure varying from 18 to 20 pounds. The preparation of sterile beet-root juice was simplified in later experiments. The juice was centrifuged for an hour, autoclaved, passed through filter paper, and again autoclaved.

Extract from Crushed Infective Beet Leafhoppers.—In the preparation of the virus extract from infective beet leafhoppers, the insects were captured with a pipette (fig. 1) and put in small vials containing beet-root juice and beet-sugar solution. The bottles were shaken so that the wings became wet and the insects were unable to fly. One gram of infective leafhoppers, or approximately 1,000 specimens, which had completed the nymphal stages on diseased beets, were used in experiments reported in this paper. The insects were transferred from the vials to a mortar by repeatedly filling a medicine dropper with the solution and forcing a stream of the liquid into the vial and then dumping the contents of the vial into the mortar. The liquid was removed from the mortar and the insects were ground with pulverized pyrex glass with a pestle in a Schultz mechanical grinder (fig. 2). To each gram of crushed leafhoppers, 99 cc of a mixture of equal parts of steam-extracted or autoclaved beet-root juice and a 5 per cent beet-sugar solution were added.

Centrifugation.—The period of centrifugation of diseased beet juice and crushed infective beet leafhoppers was usually one hour. When 1,000 to 2,000 cubic centimeters of beet juice was required, the centrifugation speed was 2,000 revolutions per minute, and with smaller quantities of juice the speed was 3,500 revolutions per minute. When the Sharples supercentrifuge was used at a speed of 40,000 revolutions per minute, the juice from the blades, petioles, blades and petioles combined, or beet root was first centrifuged at 2,000 revolutions per minute to throw down fragments of tissues that would otherwise clog the feed nozzle of the supercentrifuge.
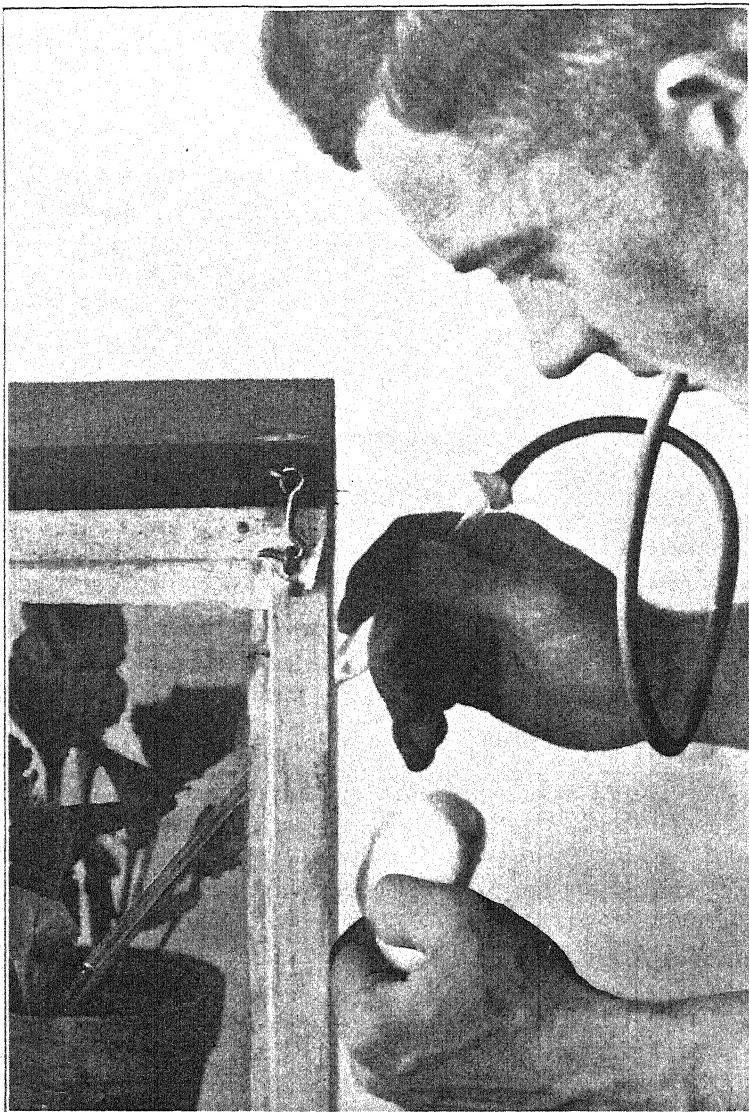
Fig. 1. Method of removing beet leafhoppers from a cage with a 10-cc pipette. By inhaling a breath of air through the rubber tube, the operator may draw the leafhoppers into the bulb of the pipette, and by exhaling he may expel them from the pipette into a vial. A piece of silk bolting covers the opening between the pipette and the rubber tubing.

Filtration.—In the filtration experiments the diseased beet juice or feeding solution containing the virus extract from the infective beet leafhoppers was centrifuged and then filtered through a coarse Berkefeld (V) or Mandler preliminary candle and refiltered through a fine Berkefeld (W), fine Mandler, or Chamberland filter. It was usually impossible to transmit curly top whenever bacterial growth developed in the filtrate owing to faulty filters or accidental aerobic contamination, and hence such preparations were not used.
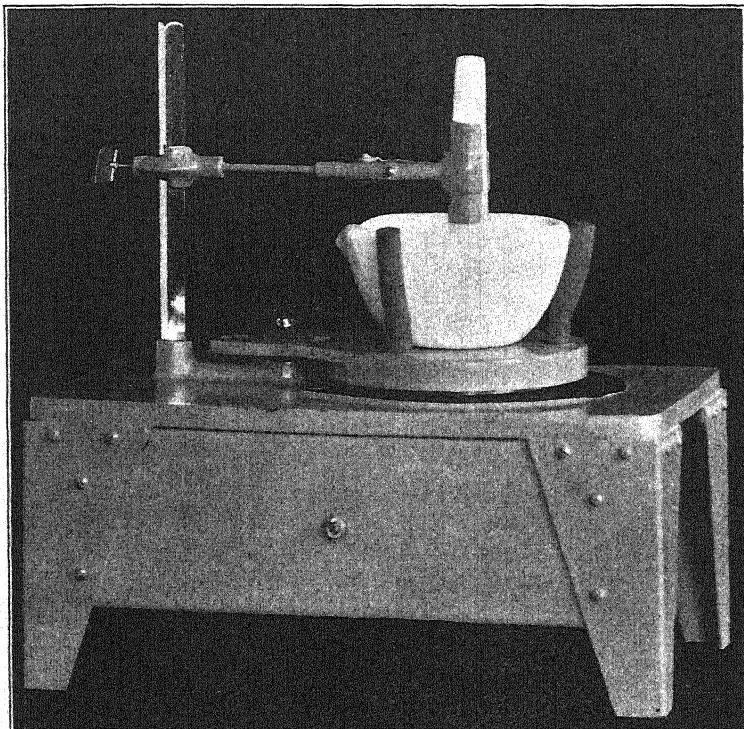
Fig. 2. Schultz electric mechanical grinder, showing mortar used in grinding beet leaves and beet leafhoppers with pulverized pyrex glass.

Feeding Beet Leafhoppers on Virus Extracts.—The methods by which the beet leafhopper can be induced to acquire the virus from virus extracts have been previously described by Carter,[9, 10, 11] Severin and Swezy,[55] and Severin.[53] The equipment for feeding noninfective nymphs consisted of a small petri or Esmarch dish (50 by 10 mm) containing about 17 cubic centimeters of the virus extract. In some experiments a stender dish (50 by 25 mm) containing about 50 cubic centimeters of the solution was used. In our early work the dish was covered

with fishskin but in later experiments Baudruche[4] transparent capping skins marked 1–A and 1–B were used. The dish was placed directly in front of the glass in a small cylindrical cage ($4\frac{3}{4}$ by $5\frac{3}{8}$ inches) covered with black sateen (fig. 3).

Feeding Period.—The noninfective nymphs were usually fasted for about 2 hours and then fed for a period of about 6 hours on unfiltered virus extracts. In the filtration experiments the insects were kept without food in empty cages during the morning, fed during the afternoon and night, and removed during the next morning, a feeding period of about 18 hours. Except in infectivity tests with single insects compared
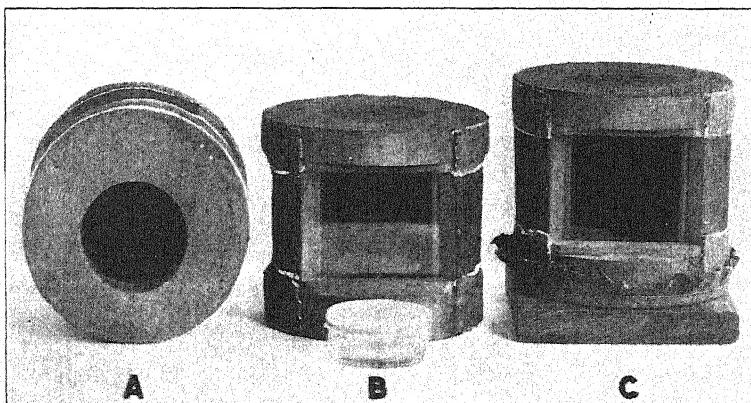
Fig. 3. Cages covered with black sateen used in feeding nymphs on virus extracts: *A*, bottom of cage showing hole off center toward the glass; *B*, stender dish covered with transparent capping skin within the cage, and also stender dish outside of cage showing height equal to that of the circular bottom board of cage; *C*, bottom of cage covered with denim and resting on a square board. The stender dish is placed directly in front of the glass in the cage and the nymphs attracted to the light come to rest on the membrane and feed.

with larger numbers, the number of nymphs used in each cage was about 100, no accurate counts being made of the number of insects. Sometimes several feeding experiments were made with each preparation. At the end of the feeding period each lot of nymphs was divided among 3 healthy beet seedlings enclosed in cages, where they remained for a period of 5 days. At the end of this time the insects were removed and the beets placed in insect-proof cages, where they were kept for a period of 3 months, if curly-top symptoms did not develop within the usual period of 10 days to 2 weeks.

Use of Noninfective Beet Leafhoppers Instead of Mechanical Inoculation.—Curly top is not readily transmitted by mechanical inoculation

[4] Baudruche capping skins are manufactured by Paul Troeder, Belleville, New Jersey.

and hence it was decided to test infectivity by feeding previously non-infective beet leafhoppers on the virus extract from diseased beets and infective leafhoppers. Severin[49] demonstrated that 6 of 72 beets and 3 of 28 beets inoculated in the crown with juice extracted from diseased leaves and beet roots respectively, developed curly top. Carsner and Stahl[8] obtained a few cases of curly top by inoculating a considerable number of healthy beets.

The results of inoculating healthy beet seedlings with curly top by means of previously noninfective leafhoppers fed on filtered and unfiltered juice from diseased beet roots have been published by Severin and Swezy.[55] They found when feeding previously non-infective nymphs at daily intervals on the filtered juice from curly-top beet roots that on the first day 67.8 to 76.1 per cent of the beets to which the insects were transferred became infected; on the second day the percentage of infection was 26.6 to 40.0, and on the third day 7.6 to 10.0 per cent. Similar tests with centrifuged, unfiltered diseased beet-root juice gave infections as follows : first day 52.9 per cent ; second day 33.3 per cent ; and third day none. Tests with the sediment after centrifugation gave 50 per cent infections the first day and none thereafter.

INOCULATION EXPERIMENTS WITH DISEASED BEET JUICE

Extracts from Leaves.—In the first experiment previously noninfective nymphs were fed on the extracted juice from the blades, petioles, and blades and petioles combined. The beet roots were too small to extract the quantity of juice required for the feeding equipment. The juice was expressed from the inner or youngest leaves showing symptoms of the disease from many small beets experimentally infected with curly top in the greenhouse. The leaves were ground in a food chopper and the juice was strained through several layers of cheesecloth. Some of the leaf juice was centrifuged for an hour and filtered through coarse and fine candles. The candles were changed frequently in filtering the juice since the pores became clogged, apparently by the chloroplasts.

From unfiltered juice of the blades, 30 beets were inoculated ; from unfiltered petiole juice, 58 plants were inoculated ; and from unfiltered juice of the leaves and petioles combined, 12 plants were inoculated. From filtrate of the blades, 9 beets were inoculated ; from filtrate of the petioles, 90 beets were inoculated ; and from filtrate of petioles and blades combined, 15 beets were inoculated. No infections resulted in any of these tests. Under natural conditions the beet leafhoppers obtain the

curly-top virus by feeding on the blades and petioles of small diseased beets such as those used for the extracts. A high mortality of the nymphs occurred during prolonged periods of feeding on the juices expressed from the leaves.

TABLE 1

INOCULATIONS OF HEALTHY BEET SEEDLINGS WITH CURLY TOP BY MEANS OF NYMPHS FED ON DILUTED JUICE EXTRACTED FROM LEAVES OF SMALL CURLY-TOP BEETS

Preparation No.	Diluted with equal parts of autoclaved beet-root juice and 5 per cent sugar solution		Diluted with 5 per cent sugar solution	
	Beets inoculated	Beets infected	Beets inoculated	Beets infected
Juice from blades				
1	12	1	12	3
2	6	0	6	0
3	6	0	6	0
Total	24	1	24	3
Percentage	....	*4.2*	....	*12.5*
Juice from petioles				
4	9	2	6	0
5	6	0	9	0
6	6	3	6	1
Total	21	5	21	1
Percentage	....	*23.8*	....	*4.8*
Juice from blades and petioles combined				
7	3	0	3	1
8	9	3	9	0
9	12	8	12	3
Total	24	11	24	4
Percentage	....	*45.8*	....	*16.7*

Results summarized according to number of preparations tested and found infectious

Source of preparation	Diluted with equal parts of autoclaved beet-root juice and 5 per cent sugar solution		Diluted with 5 per cent sugar solution	
	Tested	Infectious	Tested	Infectious
Blades	3	1	3	1
Petioles	3	2	3	1
Blades and petioles combined	3	2	3	2

In the second experiment diseased blades, petioles, or blades and petioles combined were submerged before grinding in a solution in a mortar containing either 50 cc of autoclaved beet-root juice and 50 cc of 5 per cent beet sugar dissolved in sterile distilled water or 100 cc of a 5 per cent beet-sugar solution. The leaves were sometimes ground with fine sand in a mortar or with pulverized pyrex glass in a Schultz mechanical grinder. Beet sugar was added to the feeding solution because it is a favorable food for the nymphs and reduces the mortality. For inoculum

TABLE 2

INOCULATIONS OF HEALTHY BEET SEEDLINGS BY MEANS OF NYMPHS FED ON BEET-
LEAF AND ROOT JUICE FROM EACH OF FIVE LARGE DISEASED BEETS

Diseased beet No.	Juice from blades		Juice from petioles		Juice from blades and petioles combined		Juice from beet roots	
	Beets inocu-lated	Beets in-fected	Beets inocu-lated	Beets in-fected	Beets inocu-lated	Beets in-fected	Beets inocu-lated	Beets in-fected
1	3	2	3	0	3	0	3	2
2	3	0	3	0	3	0	3	2
3	3	0	3	0	3	0	3	3
4	3	0	3	0	3	0	3	0
5	3	0	3	0	3	0	3	0
Total	15	2	15	0	15	0	15	7
Percentage	....	13.3	....	0.0	....	0.0	....	46.7

Results summarized according to number of preparations tested and found infectious

	Juice from blades		Juice from petioles		Juice from blades and petioles combined		Juice from beet roots	
	Tested	Infectious	Tested	Infectious	Tested	Infectious	Tested	Infectious
	5	1	5	0	5	0	5	3

in each test the inner or youngest leaves showing symptoms of the disease were removed from 30 small beets experimentally infected with curly top in the greenhouse. The results are shown in table 1.

According to table 1, the percentage of infections was higher with diluted juice expressed from the blades and petioles combined than with the extracts from the blades or from the petioles. The summary in table 1 shows the number of extractions found to be infectious. The results seem to indicate that oxidation was a factor in the inactivation of the virus in the previous experiment, since in this experiment the leaves were submerged in the feeding solution in the process of extracting the juice.

TABLE 3

INOCULATIONS OF HEALTHY BEET SEEDLINGS WITH CURLY TOP BY MEANS OF NYMPHS FED ON CENTRIFUGED DISEASED BEET-LEAF AND ROOT JUICE

Preparation No.	Juice from blades		Juice from petioles		Juice from blades and petioles combined		Juice from beet roots	
	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected
Centrifuged 3,500 r.p.m.								
1	6	1	6	0	15	1	9	3
2	12	7	12	0	9	0	9	7
3	6	0	6	1	9	0	6	5
4	6	0	6	0	6	0	6	0
5	6	0	6	0	6	2	6	6
6	6	0	6	0	6	0	9	8
Total	42	8	42	1	51	3	45	29
Percentage	....	19.0	....	2.4	....	5.9	....	64.4
Supercentrifuged 40,000 r.p.m.								
1	12	3	12	3	12	0	12	10
2	9	0	9	0	9	0	9	5
3	9	0	9	0	9	0	9	2
4	9	1	9	0	9	0	9	3
5	9	0	9	0	9	0	9	1
6	9	0	9	0	9	0	9	3
Total	57	4	57	3	57	0	57	24
Percentage	....	7.0	....	5.3	....	0.0	....	42.1
Supercentrifuged 40,000 r.p.m.*								
1	12	0	12	1	12	0	12	11
2	9	3	9	0	9	0	27	10
Total	21	3	21	1	21	0	39	21
Percentage	....	14.3	....	4.8	....	0.0	....	53.8

Results summarized according to number of preparations tested and found infectious

Centrifugation	Juice from blades		Juice from petioles		Juice from blades and petioles combined		Juice from beet roots	
	Tested	Infectious	Tested	Infectious	Tested	Infectious	Tested	Infectious
3,500	6	2	6	1	6	2	6	5
40,000	6	2	6	1	6	0	6	6
40,000*	2	1	2	1	2	0	2	2

* Leaf and root juice which remained in supercentrifuge after centrifugation in preparations 1 and 2.

Extract from Beet Root.—In the first two experiments no tests were made with root juice since the beets were too small to yield a sufficient quantity of juice, and hence in the third experiment large diseased beets removed from the field were used. The juice was expressed from the blades, petioles, blades and petioles combined, and beet roots from each of 5 large beets in an advanced stage of the disease. The results of inoculating healthy beet seedlings by means of previously noninfective nymphs which were fed on the extracts are indicated in table 2.

According to table 2 infections were obtained from extracts of diseased blades and root of beet No. 1, from the roots of beets Nos. 2 and 3, but no infections were obtained with root juice expressed from beets 4 and 5. The summary in table 2 shows that 1 of 5 preparations from the blades and 3 of 5 preparations from the beet roots were infectious. No infections were obtained with preparations from the petioles or with blades and petioles combined.

Centrifuged Beet Juice.—In the fourth experiment the extracts from blades, petioles, blades and petioles combined, and roots of large beets removed from the field were centrifuged for 1 hour at 3,500 revolutions per minute and other portions of each extract were supercentrifuged at 40,000 revolutions per minute. Supercentrifugation of leaf juices removed most of the chloroplasts. The results of inoculating healthy beet seedlings with curly top by means of previously noninfective nymphs fed on the centrifuged and supercentrifuged beet-leaf and root juices are shown in table 3.

That the virus can be more readily transmitted with centrifuged and supercentrifuged beet-root juice than with leaf juice seems clearly indicated in table 3. The summary in table 3 shows no marked difference in the number of preparations found infectious with centrifuged and with supercentrifuged beet-root and leaf juices.

Diluted Centrifuged Beet Juice.—In the fifth experiment extracts from diseased leaves and beet roots were diluted and then centrifuged for 1 hour at 3,500 revolutions per minute. Four different diluents were used, in each case equal parts of diseased beet juice and diluents being mixed. The diluents were: (1) autoclaved filtered beet-root juice; (2) equal parts of autoclaved filtered beet-root juice and a 5 per cent solution of beet sugar; (3) a 5 per cent solution of beet sugar; and (4) sterile distilled water. The infections obtained are indicated in table 4.

In two tests indicated in table 4 the infection with diluted diseased blade juice was higher, 66.7 per cent as compared with 53.3 per cent with diluted diseased beet-root juice. All other percentages with diluted blade, petiole, and blade and petiole juice combined were lower than with the same dilution of diseased beet-root juice. The summary in table

TABLE 4

INOCULATIONS OF HEALTHY SUGAR BEET SEEDLINGS WITH CURLY TOP BY MEANS OF NYMPHS FED ON DILUTED DISEASED BEET-LEAF AND ROOT JUICE

Preparation No.	Juice from blades		Juice from petioles		Juice from blades and petioles combined		Juice from beet roots	
	Beets inocu-lated	Beets in-fected	Beets inocu-lated	Beets in-fected	Beets inocu-lated	Beets in-fected	Beets inocu-lated	Beets in-fected
Equal parts of diseased juice and autoclaved filtered beet-root juice								
1	6	4	6	0	6	0	6	1
2	6	1	6	0	6	0	6	1
3	6	4	6	1	6	2	6	6
4	6	0	6	0	6	0	6	3
Total	24	9	24	1	24	2	24	11
Percentage	....	37.5	....	4.2	....	8.3	....	45.8
Equal parts of diseased juice and a mixture of equal parts of autoclaved beet-root juice and a 5 per cent sugar solution								
1	3	3	3	0	3	0	6	4
2	3	1	3	0	3	0	9	4
Total	6	4	6	0	6	0	15	8
Percentage	....	66.7	....	0.0	....	0.0	....	53.3
Equal parts of diseased juice and a 5 per cent sugar solution								
1	3	1	3	0	3	0	9	7
2	3	0	3	0	3	0	6	6
Total	6	1	6	0	6	0	15	13
Percentage	....	16.7	....	0.0	....	0.0	....	86.7
Equal parts of diseased juice and distilled water								
1	6	0	6	1	6	0	6	1
2	6	0	6	2	6	0	6	2
3	6	0	6	0	6	0	6	1
4	6	0	6	0	6	0	6	4
Total	24	0	24	3	24	0	24	8
Percentage	....	0.0	....	12.5	....	0.0	....	33.3

Results summarized according to number of preparations tested and found infectious

Diluent	Juice from blades		Juice from petioles		Juice from blades and petioles combined		Juice from beet roots	
	Tested	Infectious	Tested	Infectious	Tested	Infectious	Tested	Infectious
Autoclaved fil-tered beet-root juice	4	3	4	1	4	1	4	4
Autoclaved beet-root juice and a sugar solution	2	2	2	0	2	0	2	2
Sugar solution	2	1	2	0	2	0	2	2
Distilled water	4	0	4	2	4	0	4	4

4 shows that 6 of 12 preparations of blade juice, 3 of 12 preparations of petiole juice, 1 of 12 preparations of blade and petiole juice combined, and all 12 preparations from beet roots were infectious. The dilution tests, however, do not reveal an explanation for the difference in results obtained with the extracts from the leaves and beet root.

AGING OF VIRUS IN BEET-ROOT JUICE

In Extracted, Centrifuged, and Supercentrifuged Preparations.— Tests were made to determine whether the curly-top virus in diseased beet-root juice was inactivated more rapidly by exposure to the air at room temperature in a small petri or Esmarch dish (50 by 10 mm) containing 17 cc or in a stender dish (50 by 25 mm) containing 50 cc. In each test the extract from large diseased beet roots was divided into 3 portions; one part was not centrifuged; another portion was centrifuged for a period of 1 hour at 3,500 revolutions per minute; and the last part was supercentrifuged at 40,000 revolutions per minute, using the coarse nozzle once and the fine nozzle twice. The results obtained are shown in table 5.

An inactivation of the virus occurred after the beet-root juice was exposed to the air at room temperatures for a period of 72 hours, as is shown in table 5. In some of the tests, except with supercentrifuged beet-root juice, the virus extract became thick and jelly-like owing to bacterial growth, within 16 to 76 hours, according to temperature. The lowest percentage of infection was obtained with extracted beet-root juice in an Esmarch dish containing 17 cubic centimeters.

In Aerobic and Anaerobic Filtrates.—The longevity of the curly-top virus was determined by aging the filtrate prepared from the juice of diseased beet roots under aerobic and anaerobic conditions. The aerobic filtrate was kept in sterile test tubes plugged with cotton, while in other test tubes the surface of the filtrate was capped with a mixture of equal parts of hot melted paraffin and crude vaseline to prevent oxidation and provide partially anaerobic conditions. Previously noninfective nymphs were fed at daily intervals on the aerobic and partially anaerobic filtrates and then the insects were transferred to 3 healthy beet seedlings in each test. Table 6 shows the results obtained.

According to table 6 the transmission of curly top varied as follows: aerobic filtrate 13.3 to 66.7 per cent; anaerobic filtrate 19.0 to 70.8 per cent. The highest percentages of infections were obtained during the first 2 days with the aerobic filtrate and during the first 3 days with the anaerobic filtrate.

Table 6 shows that the virus was recovered from 4 of 5 aerobic preparations on each of the first, second, and third days; from 3 of 5 on the fourth and fifth days; from none on any of the succeeding days except from 1 of 5 on the eighth day. With the anaerobic filtrate the virus was reclaimed from 5 of 5 preparations tested on the first, second, and third

TABLE 5

EFFECT OF EXPOSING VIRUS EXTRACT FROM DISEASED BEET ROOTS TO THE AIR AT ROOM TEMPERATURE

Number of hours exposed to air	Extracted beet-root juice				Centrifuged beet-root juice 3,500 r.p.m. 1 hour				Supercentrifuged beet-root juice 40,000 r.p.m.			
	17 cc		50 cc		17 cc		50 cc		17 cc		50 cc	
	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected
2	....	....	3	2	....	....	3	2	....	....	....	....
3	....	....	3	1	....	....	3	2	....	....	....	...
4	....	....	6	3	....	....	6	3	....	....	3	0
5	....	....	3	0	....	...	3	0	....	....	....	....
6	....	....	6	3	....	....	6	4	....	....	3	3
7	....	....	3	0	....	...	3	1	....	....	....	....
8	....	....	6	2	....	....	6	4	....	....	3	0
9	...	....	3	1	...	....	3	0	....	....	....	....
10	3	0	6	0	....	....	3	0	....	....	3	0
12	3	0	3	3	....	....	3	1	....	....	3	1
14	3	0	3	1	....	....	3	1	....	....	3	1
16	6	0	6	1	3	0	6	0	3	1	6	4
17	6	1	6	3	6	3	6	3	6	4	6	6
18	3	0	3	0	3	0	6	0	3	0	3	0
19	6	3	6	1	6	4	6	3	6	1	6	1
20	3	0	3	1	3	0	3	0	3	1	3	0
21	6	3	6	3	6	3	6	3	6	3	6	3
22	3	0	3	0	3	0	3	0	3	1	3	0
23	6	1	6	3	6	4	6	2	6	4	6	6
24	3	0	3	0	3	0	3	0	3	0	3	1
25	6	3	6	1	6	3	6	1	6	2	6	2
26	6	4	6	4	6	4	6	3	6	2	6	0
28	6	3	6	3	6	2	6	4	6	2	6	3
30	6	0	6	3	6	3	6	0	6	3	6	3
32	6	1	6	4	6	1	6	1	6	3	6	1
40	6	0	6	0	6	0	6	0	6	0	6	1
42	6	0	6	0	6	0	6	0	6	0	6	0
44	6	0	6	1	6	0	6	0	6	0	6	1
46	6	0	6	0	6	0	6	0	6	0	6	0
48	12	3	12	1	12	3	12	2	12	3	12	0
50	6	0	6	1	6	0	6	0	6	1	6	1
52	6	0	6	0	6	0	6	0	6	0	6	0
54	3	0	3	0	3	0	3	1	3	0	3	0
72	6	0	6	0	6	0	6	0	6	0	6	0
74	6	0	6	0	6	0	6	0	6	0	6	0
76	6	0	6	0	6	0	6	0	6	0	6	0
Total..........	150	22	186	46	138	30	186	41	138	31	159	38
Percentage....	....	14.7	....	24.7	....	21.7	....	22.0	....	22.5	....	23.9

day; from 4 of 5 on the fourth day; 2 of 5 on the fifth day; 4 of 5 on the sixth day; 5 of 5 on the seventh day; 3 of 4 on the eighth day; and 1 of 2 on the ninth day.

TABLE 6

DAILY INOCULATIONS OF HEALTHY BEET SEEDLINGS WITH CURLY TOP BY MEANS OF NYMPHS FED ON AEROBIC AND PARTIALLY ANAEROBIC FILTRATES PREPARED FROM JUICE EXTRACTED FROM DISEASED BEET ROOTS

Age of preparation, days	Preparation No. 1		Preparation No. 2		Preparation No. 3		Preparation No. 4		Preparation No. 5		Totals			Number of preparations	
	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Per cent	Tested	Infectious
Aerobic filtrate															
1	3	2	3	0	3	3	3	2	3	2	15	9	60.0	5	4
2	3	0	3	2	3	3	3	3	3	2	15	10	66.7	5	4
3	3	2	3	0	3	3	3	1	3	1	15	7	46.7	5	4
4	3	2	3	0	3	3	3	0	3	3	15	8	53.3	5	3
5	3	1	3	0	3	2	3	0	3	2	15	5	33.3	5	3
6	3	0	3	0	3	0	3	0	3	0	15	0	0.0	5	0
7	3	0	3	0	3	0	3	0	3	0	15	0	0.0	5	0
8	3	0	3	0	3	0	3	2	3	0	15	2	13.3	5	1
9	3	0	3	0	3	0	3	0	3	0	15	0	0.0	5	0
10	3	0	3	0	3	0	3	0	3	0	15	0	0.0	5	0
Anaerobic filtrate															
1	6	5	6	3	6	4	3	3	3	2	24	17	70.8	5	5
2	6	2	6	1	6	5	3	2	3	3	22	13	59.1	5	5
3	6	2	6	1	3	3	3	3	3	3	21	12	57.1	5	5
4	3	1	6	2	6	3	3	3	3	0	21	9	42.9	5	4
5	3	0	6	0	6	2	3	2	3	0	21	4	19.0	5	2
6	3	2	6	2	6	3	3	0	3	2	21	9	42.9	5	4
7	3	1	6	2	6	2	3	2	3	3	21	10	47.6	5	5
8	3	2	6	0	....	....	3	3	3	2	15	7	46.7	4	3
9	3	3	3	0	....	....	....	....	....	....	6	3	50.0	2	1
10	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....

In later experiments weekly inoculations were made with partially anaerobic filtrates prepared from supercentrifuged diseased beet-root juice, and infections were obtained at the end of 5 weeks as shown in table 7.

In Filtrate Prepared from Diluted Supercentrifuged Preparations.— An experiment was conducted with partially anaerobic filtrates prepared from supercentrifuged diseased beet-root juice. In one test the beet-root juice was diluted with equal parts of sterile distilled water and in another test the extract was not diluted. Each was supercentrifuged

at 40,000 revolutions per minute. The filtrate in test tubes was capped with equal parts of paraffin and vaseline. Table 7 shows the results of weekly inoculations during a period of 10 weeks.

TABLE 7

WEEKLY INOCULATIONS OF HEALTHY BEET SEEDLINGS WITH
CURLY TOP BY MEANS OF NYMPHS FED ON PARTIALLY
ANAEROBIC FILTRATES PREPARED FROM DILUTED
AND UNDILUTED SUPERCENTRIFUGED DIS-
EASED BEET-ROOT JUICE

Age of filtrate, weeks	Filtrate prepared from undiluted juice		Filtrate prepared from juice diluted 1:1 with distilled water	
	Beets inoculated	Beets infected	Beets inoculated	Beets infected
1	6	6	6	2
2	3	2	3	2
3	3	0	3	3
4	3	1	3	0
5	3	1	3	0
6	3	0	3	0
7	3	0	3	0
8	3	0	3	0
9	3	0	3	0
10	3	0	3	0

According to table 7 infections were obtained with the diluted partially anaerobic filtrate at the end of 3 weeks and with the partially anaerobic filtrate at the end of 5 weeks. The age of the virus was not increased in the partially anaerobic filtrate prepared from the diluted supercentrifuged diseased beet-root juice as compared with the partially anaerobic filtrate prepared from the undiluted supercentrifuged root juice.

Effect of pH on Aging Under Anaerobic Conditions.—The effect of aging on the curly-top virus was determined with the filtrate prepared from diseased beet-root juice placed in anaerobic jars. The extract was adjusted to a pH range from 6.4 to 2.9 and then filtered through coarse and refiltered through fine Berkefeld candles. Anaerobic conditions were produced by exhausting the air in the jars with hydrogen, and by the use of pyrogallolic acid placed on the bottom of the jars. It is questionable whether strictly anaerobic conditions prevailed in the jars. The filtrates used as a control were fed to previously noninfective nymphs a few hours after the pH was adjusted. Table 8 shows the results obtained during a period of 100 days.

In beet-root juice adjusted to pH 3.5 the virus was apparently inactivated the first time it was tested at the end of 7 days, while with the same juice at pH 5.0 and pH 6.0 the virus was active after 100 days.

TABLE 8

EFFECT OF pH ON AGING UNDER PARTIALLY ANAEROBIC CONDITIONS

Age of filtrate, days	Prepa-ration No.	pH 6.0*		pH 5.0		pH 4.0		pH 3.5		pH 3.0†	
		Beets inocu-lated	Beets in-fected	Beets inocu-lated	Beets in-fected	Beets inocu-lated	Beets in-fected	Beets inocu-lated	Beets in-fected	Beets inocu-lated	Beets in-fected
0	1	3	3	3	3	3	3	....	....	3	0
	2	3	1	3	3	3	3	....	....	3	0
	3	3	1	3	3	3	1	....	....	3	0
3	1	6	2	6	5	6	0	....	....	6	0
	2	3	0	3	1	3	0	....	....	3	0
4	3	3	2	6	0	6	1	....	....	6	0
7	1	6	0	6	5	6	0	....	....	6	0
	2	6	6	6	6	....	....	....	....	....	....
	3	3	1	3	0	3	0	....	....	3	0
	4	3	1	3	2	....	....	3	0	....	....
10	2	3	0	6	3	6	0	....	....	....	....
14	4	3	3	3	3	....	....	3	0	....	....
22	4	3	2	3	2	....	....	3	0	....	....
34	4	6	5	3	2	....	....	6	0	....	....
71	4	3	2	3	3	....	....	3	0	....	....
100	4	6	3	6	4	....	....	3	0	....	....

* Preparation No. 1 had a pH of 6.4, No. 2 a pH of 6.3, and Nos. 3 and 4 a pH of 6.0.
† Preparation Nos. 1 and 2 had a pH of 2.9, No. 3 a pH of 3.0.

CULTIVATION OF VIRUS OUTSIDE OF LIVING PLANT

Olitisky[43, 44] came to the conclusion that the virus of tobacco and tomato mosaic is a living, multiplying, microbic body which can be cultivated and is capable of propagating itself through many generations in an artificial medium. He obtained infections with the twelfth subculture representing a dilution magnitude of 4×10^{-16} whereas the dilution limit was $10.^{-6}$

Mulvania,[41] Purdy,[46] Goldsworthy,[21] Smith,[56] and Grainger[23] have tried to repeat this experiment but without success.

Tests were made to determine whether the curly-top virus could be cultivated in a feeding solution under anaerobic conditions. The feeding solution consisted of 300 cc of sterile beet-root juice to which was added 50 cc of a 2 per cent solution of beet sugar and 50 cc of a 2 per cent solution of soluble starch. After the test tubes containing the filtered diseased beet-root juice adjusted to pH 6.0 and pH 5.0 were removed from the anaerobic jar at the end of 100 days in the previous experiments, 1 loop or 1 cc was transplanted in 16 cc of the feeding solution. The first

transplants were incubated in an anaerobic jar for a period of 10 days and the second transplants for a period of 10 additional days. Noninfective nymphs after feeding on the first and second transplants failed to transmit curly top to healthy beet seedlings as indicated in table 9. Aging the virus under anaerobic conditions probably reduces its virulence.

TABLE 9

ATTEMPT TO CULTIVATE CURLY-TOP VIRUS IN A FEEDING SOLUTION UNDER ANAEROBIC CONDITIONS

Preparation No.	Transplant	Quantity of filtrate transplanted	Period of incubation, days	pH 6		pH 5	
				Beets inoculated	Beets infected	Beets inoculated	Beets infected
1	First	1 loop	10	3	0	3	0
	Second	1 loop	20	3	0	3	0
2	First	1 cc	10	3	0	3	0
	Second	1 cc	20	3	0	3	0
3	First	1 cc	10	3	0	3	0
	Second	1 cc	20	3	0	3	0

RESISTANCE OF VIRUS TO DRYING IN PLANT TISSUES AND IN INFECTIVE BEET LEAFHOPPERS

The curly-top virus was inactivated in dried diseased beet pulp and beet roots. In one experiment 40 diseased beet roots were ground in a meat grinder and the pulp was slowly dried in the greenhouse for a period of 5 weeks and in the headhouse where the light was less intense for a period of 7 weeks. In another experiment the beet roots of 20 plants, in an advanced stage of the disease, were dried in the greenhouse for a period of 7 weeks. In both experiments the dried plant tissue was steeped in equal parts of steam-extracted beet-root juice and sterile distilled water containing 5 per cent beet sugar. Noninfective nymphs after feeding on the filtered and unfiltered beet-root extracts failed to transmit curly top to healthy beet seedlings.

Dried infective beet leafhoppers were pulverized in a mortar and steeped in a feeding solution similar to that used in the preceding experiment. Noninfective nymphs after feeding on the filtered and unfiltered extract, were transferred to healthy beet seedlings, but no curly top developed.

INACTIVATION OF VIRUS WITH JUICES FROM IMMUNE HOST PLANT

It has been found that it is not possible to transmit curly top to any plant of the Gramineae, or grass family. Alameda or Mammoth sweet corn *(Zea mays)* is a favorable food plant of the beet leafhopper but is unfavorable to the curly-top virus. It was decided to express the juice from healthy sweet corn plants and determine whether the curly-top virus in beet-root juice was inactivated in the extract from sweet-corn plants. The sweet-corn and beet-root juices were centrifuged for 1 hour at 3,500 revolutions per minute except in one test indicated in table 10, in which corn juice was supercentrifuged at 40,000 revolutions per minute. Various dilutions were used and a period of 2, 4, or 6 hours elapsed before exposing the previously noninfective nymphs to the feeding solution. In two tests the nymphs were exposed to the feeding solution immediately after the dilutions were made. The controls were diluted with sterile distilled water. The results are indicated in table 10.

It is evident from table 10 that infections were obtained with diseased beet-root juice diluted with sweet-corn juice as follows: 4:1, 2:1, 1:1, and 1:2. No infections were obtained with dilutions of 1:50, 1:100, and 1:200 when a period of 2, 4, and 6 hours elapsed before exposing the previously noninfective nymphs to the feeding solution.

Starrett[58] found in her studies on the transmission of curly top to *Oxalis stricta* that the acidity for normal leaves and young stems of the species is pH 2.45, and for the young leaves it is pH 2.23. The juice extracted from corn plants was pH 5.5. In all probability there are other factors in corn juice which inactivate the curly-top virus.

PURIFICATION OF VIRUS

McKinney[40] and Brewer, Kraybill, Samson, and Gardner[6] have attempted purification methods with mosaic diseases. The best success was obtained with the residue from supercentrifuged juice.

Similar supercentrifuging tests were made with extracts from the blades and petioles combined; blades, petioles, and beet root combined; and beet root. The leaves and beet roots were ground in a food chopper and the juices were expressed through muslin. The juice was diluted with an equal volume of distilled water and passed through a supercentrifuge at a speed of 40,000 revolutions per minute. The diluted juice was supercentrifuged three times, using the coarse nozzle the first time and the fine nozzle the second and third times. The gummy residue was

scraped from the bowl of the supercentrifuge, resuspended in distilled water equal to the original volume of the beet juice, and passed through the supercentrifuge using the fine nozzle. The second residue formed in

TABLE 10

INOCULATIONS OF HEALTHY BEET SEEDLINGS WITH CURLY TOP BY MEANS OF NYMPHS FED ON DISEASED BEET-ROOT JUICE DILUTED WITH JUICE EXPRESSED FROM SWEET-CORN PLANTS

Experiments with lower dilutions

Period elapsed before feeding nymphs, hours	Preparation No.	Diluent	Ratio of diseased beet-root juice to diluent									
			4:1		2:1		1:1		1:2		1:4	
			Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected
0	1	Water	3	2	3	2	3	3	....	....	....	....
	1	Corn juice	3	3	3	3	3	2	....	....	....	....
	2	Corn juice	3	0	3	0	3	0	....	....	....	....
	3	Corn juice	3	3	3	2	3	0	....	....	....	....
	4	Water	....	....	....	....	3	1	3	2	3	2
	4	Corn juice	....	....	....	....	3	0	3	0	3	1
2	4	Water	....	....	....	....	3	2	3	0	3	3
	4	Corn juice	....	....	....	....	3	0	3	0	3	1
4	4	Water	....	....	....	....	3	2	3	1	3	1
	4	Corn juice	....	....	....	....	3	0	3	1	3	0

Experiments with higher dilutions

Period elapsed before feeding nymphs, hours	Preparation No.	Diluent	Ratio of diseased beet-root juice to diluent							
			1:1		1:50		1:100		1:200	
			Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected
2	1	Water	3	2	3	0	3	1	3	0
	1	Corn juice*	3	1	3	0	3	0	3	0
4	2	Water	3	1	3	1	3	2	3	0
	2	Corn juice*	3	0	3	0	3	0	3	0
2	3	Water	....	....	3	3	3	1	3	1
	3	Corn juice	....	....	3	0	3	0	3	0
4	4	Water	....	....	3	1	3	1	3	0
	4	Corn juice	....	....	3	0	3	0	3	0
6	5	Water	....	....	3	1	3	1	3	0
	5	Corn juice	....	....	3	0	3	0	3	0

* Corn juice supercentrifuged 40,000 r.p.m.

the bowl was discarded. Aluminum gel adjusted to pH 5.8 and 6.2 was added to the supercentrifuged liquid and the mixture was filtered through coarse and fine candles. The results of feeding previously non-infective nymphs on the liquid after each supercentrifugation and on the filtrate are shown in tables 11 and 12.

TABLE 11

INOCULATIONS OF HEALTHY BEET SEEDLINGS WITH CURLY TOP BY MEANS OF NYMPHS
FED ON DILUTED EXTRACTS FROM DISEASED BEETS SUPERCENTRIFUGED
THREE TIMES AND FOURTH TIME WITH GUMMY RESIDUE
RESUSPENDED IN DISTILLED WATER

Source of preparation	Preparation No.	Number of supercentrifugations							
		1		2		3		4	
		Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected
Blades and petioles	1	3	0	3	1	3	0	3	0
	2	3	1	3	0	3	0	3	1
	—	—		—		—		—	
	Total	6	1	6	1	6	0	6	1
	Percentage	....	*16.7*	....	*16.7*	....	*0.0*	....	*16.7*
Blades, petioles, and beet roots	3	3	0	3	1	3	1	3	1
	4	3	1	3	0	3	0	3	0
	5	3	1	3	2	3	1	3	0
	—	—		—		—		—	
	Total	9	2	9	3	9	2	9	1
	Percentage	....	*22.2*	....	*33.3*	....	*22.2*	....	*11.1*
Beet roots	6	3	1	3	1	3	1	3	1
	7	3	0	3	1	3	3	3	1
	8	3	0	3	1	3	0	3	1
	9	3	1	3	1	3	2	3	2
	10	3	1	3	0	3	1	3	0
	—	—		—		—		—	
	Total	15	3	15	4	15	7	15	5
	Percentage	....	*20.0*	....	*26.7*	....	*46.7*	....	*33.3*

Results summarized according to number of preparations tested and found infectious

Source of preparation	Number of supercentrifugations							
	1		2		3		4	
	Tested	Infectious	Tested	Infectious	Tested	Infectious	Tested	Infectious
Blades and petioles	2	1	2	1	2	0	2	1
Blades, petioles, and beet roots	3	2	3	2	3	2	3	1
Beet roots	5	3	5	4	5	4	5	4

According to Brewer *et al.*[6] the juice from typical tomato mosaic plants after centrifugation three times should contain relatively little virus. The gummy residue resuspended in distilled water, according to McKinney's[40] supercentrifugation tests with tobacco mosaic, contained most of the virus.

TABLE 12

INOCULATIONS OF HEALTHY BEET SEEDLINGS WITH NYMPHS FED ON FILTRATE CONTAINING A MIXTURE OF SUPERCENTRIFUGED LIQUID PREPARED FROM GUMMY RESIDUE OF DISEASED BEET-ROOT JUICE AND ALUMINUM GEL

| | Amount of aluminum gel in 100 cc of filtrate | | | | | |
| Age of filtrate | 1 cc | | 5 cc | | 10 cc | |
	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected
1 hour	....	....	6	1	6	0
1½ hours	....	....	3	0	3	0
2 hours	3	1	6	1	6	2
1 day	3	1	3	0	3	0
5 days	....	....	3	0	3	0
8 days	....	....	3	0	3	0
2 weeks	....	....	3	0	3	0
3 weeks	....	....	3	0	3	0
4 weeks	....	....	3	0	3	0
5 weeks	....	....	3	0	3	0
6 weeks	....	....	3	0	3	0

It is evident from table 11 that infections were obtained with beet extracts, with one exception, after each supercentrifugation. There was no evidence to show that an increase in the number of infections occurred with the supercentrifuged liquid obtained from the gummy residue resuspended in distilled water. No infections were obtained after the first day with the filtrate containing a mixture of supercentrifuged liquid prepared from the gummy residue and aluminum gel, as indicated in table 12.

DILUTION OF VIRUS FROM DISEASED BEET ROOTS

Three experiments were conducted in determining the tolerance to dilution of the curly-top virus in diseased beet-root juice using extracted, centrifuged, and filtered juice. Conical beakers or ordinary beakers were used in this work, and the dilutions were made with pipettes. The diluent consisted of sterile distilled water. The diluted juice was thoroughly agitated by a circular movement of the beaker and by pouring the solution back and forth in 2 beakers. A stender dish (50 by 25 mm) containing about 50 cc of diluted juice was used in the feed-

ing experiments. An undiluted control was used in each experiment. Tables 13–15 show the results obtained in the three experiments.

In Extracted Juice.—The tolerance to dilution of extracted beet-root juice was 1:100 as indicated in table 13. The percentage of infections was lower with the undiluted control than with dilutions of 1:10, 1:25,

TABLE 13

INOCULATION OF HEALTHY BEET SEEDLINGS WITH CURLY TOP BY MEANS OF NYMPHS FED ON DILUTED EXTRACTED DISEASED BEET-ROOT JUICE

Prepara-tion No.	Undiluted control		Dilutions of extracted diseased beet-root juice											
			1:10		1:25		1:50		1:100		1:200		1:300	
	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected
1	3	0	3	1	3	1	3	1	3	0	....	....	....	....
2	3	0	3	0	3	0	3	0	3	0	....	....	....	....
3	3	0	6	1	6	4	6	1	3	0	....	....	....	....
4	3	1	....	....	....	....	....	....	3	0	3	0	3	0
5	3	1	....	....	....	....	....	....	3	0	6	0	12	0
6	3	0	....	....	....	....	....	....	9	1	9	0	9	0
Total	18	2	12	2	12	5	12	2	24	1	18	0	24	0
Percentage	....	*11.1*	....	*16.7*	....	*41.7*	....	*16.7*	....	*4.2*	....	*0.0*	....	*0.0*

Results summarized according to number of preparations tested and found infectious

	Undiluted control		1:10		1:25		1:50		1:100		1:200		1:300	
			Dilutions of extracted diseased beet-root juice											
	Tested	Infectious	Tested	Infectious	Tested	Infectious	Tested	Infectious	Tested	Infectious	Tested	Infectious	Tested	Infectious
	6	2	3	2	3	2	3	2	6	1	3	0	3	0

and 1:50. A high mortality of the nymphs often occurred when fed on undiluted extracted beet-root juice used as a control. When previously noninfective nymphs failed to obtain the infective dose from a preparation in both the undiluted control and dilutions, the test of that preparation was not included in table 13; there were three such preparations with the extracted juice. On the other hand, when infections were obtained with the undiluted control and not with the dilutions or vice versa, the tests were included in the table. The summary in table 13 shows that 2 of 6 preparations of extracted diseased beet-root juice used

TABLE 14

INOCULATION OF HEALTHY BEET SEEDLINGS WITH CURLY TOP BY MEANS OF NYMPHS FED ON DILUTED, CENTRIFUGED, DISEASED BEET-ROOT JUICE

Preparation No.	Undiluted control		Dilutions of centrifuged diseased beet-root juice											
			1:10		1:25		1:50		1:100		1:200		1:300	
	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected
1	9	2	3	2	3	1	3	0	3	0	3	0	....	....
2	3	3	3	3	3	3	3	3	3	3	3	2	....	....
3	3	3	3	3	3	3	3	2	3	2	3	3	...:	....
4	3	3	....	....	....	....	....	....	3	1	3	0	3	0
5	3	3	....	....	....	....	....	....	3	1	3	2	3	1
6	3	2	....	....	....	....	....	....	3	0	3	1	3	0
7	3	3	....	....	....	....	....	....	3	2	3	0	3	1
8	6	6	....	....	....	....	....	....	3	3	3	2	3	3
Total	33	25	9	8	9	7	9	5	24	12	24	10	15	5
Percentage	....	75.8	....	88.9	....	77.8	....	55.6	....	50.0	....	41.7	....	33.3

Preparation No.	Dilutions of centrifuged diseased beet-root juice													
	1:400		1:500		1:600		1:700		1:800		1:900		1:1,000	
	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected
1	....	....	....	....	3	2	3	0	3	0	3	0	3	0
2	....	....	....	....	3	1	3	0	3	0	3	0	3	0
3	...:	...:	...:	...:	3	0	3	0	3	0	3	0	3	0
4	3	0	3	1	3	0	3	0	3	0	3	0	3	0
5	3	0	3	0	3	0	3	0	3	0	3	0	3	0
6	3	0	3	0	3	0	3	0	3	0	3	0	3	0
7	3	0	3	0	6	0	3	0	3	0	3	0	3	0
8	3	2	3	2	3	2	3	1	3	1	3	2	3	1
Total	15	2	15	3	27	5	24	1	24	1	24	2	24	1
Percentage	....	13.3	....	20.0	....	18.5	....	4.2	....	4.2	....	8.5	....	4.2

Results summarized according to number of preparations tested and found infectious

Undiluted control		Dilutions of centrifuged diseased beet-root juice											
		1:10		1:25		1:50		1:100		1:200		1:300	
Tested	Infectious	Tested	Infectious	Tested	Infectious	Tested	Infectious	Tested	Infectious	Tested	Infectious	Tested	Infectious
8	8	3	3	3	3	3	2	8	6	8	5	5	3

Dilutions of centrifuged diseased beet-root juice													
1:400		1:500		1:600		1:700		1:800		1:900		1:1,000	
Tested	Infectious	Tested	Infectious	Tested	Infectious	Tested	Infectious	Tested	Infectious	Tested	Infectious	Tested	Infectious
5	1	5	2	8	3	8	1	8	1	8	1	8	1

TABLE 15

INOCULATION OF HEALTHY BEET SEEDLINGS WITH CURLY TOP BY MEANS OF NYMPHS FED ON DILUTED, FILTERED, DISEASED BEET-ROOT JUICE

Filter candles used	Preparation No.	Undiluted control Beets inoculated	Undiluted control Beets infected	1:10 Beets inoculated	1:10 Beets infected	1:25 Beets inoculated	1:25 Beets infected	1:50 Beets inoculated	1:50 Beets infected	1:100 Beets inoculated	1:100 Beets infected
Berkefeld V, W	1	3	2	3	1	3	2	3	3	3	2
	2	3	1	3	2	3	3	3	2	3	2
	3	3	2	3	3	3	1	3	3	3	0
	4	6	1	6	6	3	1	3	1	3	1
Mandler 2 to 5, 10 to 16	5	3	0	....	....	....	....	3	0	3	0
	6	3	3	....	....	....	....	3	0	3	1
	7	3	3	....	....	....	....	3	0	3	1
Berkefeld V, W; Chamberland L 13	8	6	2	3	0	3	1	3	0	3	0
Total	....	30	14	18	12	15	8	24	9	24	7
Percentage	....	....	*46.7*	....	*66.7*	....	*53.3*	....	*37.5*	....	*29.2*

Filter candles used	Preparation No.	1:200 Beets inoculated	1:200 Beets infected	1:300 Beets inoculated	1:300 Beets infected	1:400 Beets inoculated	1:400 Beets infected	1:500 Beets inoculated	1:500 Beets infected
Berkefeld V, W	1	....	....	....	....	....	....	3	0
	2	....	....	....	....	....	....	3	0
	3	....	....	....	....	....	....	3	0
	4	3	2	3	2	3	0	3	0
Mandler 2 to 5, 10 to 16	5	3	1	3	0	3	0	3	0
	6	3	0	3	0	3	0	3	0
	7	3	0	....	....	3	0	3	0
Berkefeld V, W; Chamberland L 13	8	3	0	....	....	....	....	....	....
Total	....	15	3	9	2	12	0	21	0
Percentage	....	....	*20.0*	....	*22.2*	....	*0.0*	....	*0.0*

Results summarized according to number of preparations tested and found infectious

Undiluted control Tested	Undiluted control Infectious	1:10 Tested	1:10 Infectious	1:25 Tested	1:25 Infectious	1:50 Tested	1:50 Infectious	1:100 Tested	1:100 Infectious
8	7	5	4	5	5	8	4	8	5

Dilutions of filtrate prepared from diseased beet-root juice

1:200 Tested	1:200 Infectious	1:300 Tested	1:300 Infectious	1:400 Tested	1:400 Infectious	1:500 Tested	1:500 Infectious
5	2	3	1	4	0	7	0

as an undiluted control were infectious; 2 of 3 preparations in each dilution of 1:10, 1:25, and 1:50, and 1 of 6 preparations diluted 1:100, were infectious.

In Centrifuged Juice.—The tolerance to dilution with centrifuged diseased beet-root juice was 1:1,000 and was obtained with 49 small beets removed from the field during the spring. Infections were also obtained at intervals of 100, from 100 to 1,000 with centrifuged root juice extracted from the same 49 diseased beets as is shown in table 14. With undiluted centrifuged diseased beet-root juice used as a control 75.8 per cent of the beets were infected, whereas 11.1 per cent infections were obtained with undiluted extracted beet-root juice which was not centrifuged. According to the summary of table 14 all of the 8 centrifuged preparations used as undiluted controls were infectious while only 2 of 6 preparations not centrifuged used as undiluted controls were infectious. The summary of table 14 shows that 2 of 5 preparations with a dilution of 1:500, 3 of 8 preparations with a dilution of 1:600, and 1 of 8 preparations with each dilution of from 1:700 to 1:1,000 were infectious.

In four additional tests not listed in table 14, dilutions were made at intervals of 100 from 1,000 to 2,000,[5] and at intervals of 1,000 from 1,000 to 10,000, but no infections were obtained with 147 beets that were inoculated.

In Filtered Juice.—The tolerance to dilution of filtered diseased beet-root juice was 1:300. The number of preparations tested and found to be infectious is shown in the summary of table 15.

VIRUS EXTRACT FROM INFECTIVE BEET LEAFHOPPERS

Diluents.—Different diluents were tested with the virus extract from crushed infective beet leafhoppers. The leafhoppers were transferred from infected beets to Mammoth or Alameda sweet corn, which is immune to curly top so that the alimentary canal would not contain unchanged diseased beet juice. One gram of leafhoppers was crushed in a mortar with a pestle in a Schultz mechanical grinder, and then 99 cc of equal parts of steam-extracted beet-root juice and a 5 per cent beet-sugar solution were added. The mixture was centrifuged for 1 hour at 3,500 revolutions per minute and was then used as a stock solution for dilution. The results obtained with different diluents are indicated in table 16.

A comparison of the percentages of beets infected with the virus extract from infective beet leafhoppers diluted with different diluents in table 16, and the number of preparations found to be infectious, as given

[5] During the spring of 1933 a dilution of 1:2,000 was obtained with centrifuged diseased beet-root juice from beets removed from the field.

TABLE 16

INOCULATIONS OF HEALTHY BEET SEEDLINGS WITH CURLY TOP BY MEANS OF NYMPHS FED ON THE VIRUS EXTRACT FROM INFECTIVE BEET LEAFHOPPERS DILUTED WITH DIFFERENT DILUENTS

| Preparation No. | Ratio of virus extract of infective leafhoppers to diluent | | | | | | | | | |
| | 1:100 | | 1:200 | | 1:300 | | 1:400 | | 1:500 | |
	Beets inocu-lated	Beets in-fected	Beets inocu-lated	Beets in-fected	Beets inocu-lated	Beets in-fected	Beets inocu-lated	Beets in-fected	Beets inocu-lated	Beets in-fected
Diluted with equal parts of steam-extracted beet-root juice and a 5 per cent sugar solution										
1	6	3	6	1	....	....	....	....	....	....
2	3	0	6	2	6	2	9	0	....	....
3	3	1	....	....	....	....	9	3	12	5
4	3	0	....	....	....	....	....	....	9	1
5	6	0	9	0	15	3	3	0	....	....
Total	21	4	21	3	21	5	21	3	21	6
Percentage	....	*19.0*	....	*14.3*	....	*23.8*	....	*14.3*	....	*28.6*
Diluted with 5 per cent sugar solution										
6	6	2	6	3	....	....	....	....	....	....
7	3	3	6	6	6	5	9	1	....	....
8	3	1	....	....	....	....	9	4	12	4
9	9	2	9	1	15	4	3	0	9	0
Total	21	8	21	10	21	9	21	5	21	4
Percentage	....	*38.1*	....	*47.6*	....	*42.9*	....	*23.8*	....	*19.0*
Diluted with distilled water										
10	6	3	6	5	....	....	....	....	....	....
11	3	2	6	5	6	4	9	3	....	....
12	3	2	....	....	....	....	9	7	12	7
13	3	0	....	....	....	....	....	....	6	1
14	6	1	9	2	15	0	3	0	3	0
Total	21	8	21	12	21	4	21	10	21	8
Percentage	....	*38.1*	....	*57.1*	....	*19.0*	....	*47.6*	....	*38.1*

Results summarized according to the number of preparations tested and found infectious

| Diluent | Ratio of virus extract of infective leafhoppers to diluent | | | | | | | | | |
| | 1:100 | | 1:200 | | 1:300 | | 1:400 | | 1:500 | |
	Tested	Infec-tious	Tested	Infec-tious	Tested	Infec-tious	Tested	Infec-tious	Tested	Infec-tious
Steam-extracted beet-root juice and sugar solution	5	2	3	2	2	2	3	1	2	2
Sugar solution	4	4	3	3	2	2	3	2	2	1
Distilled water	5	4	3	3	2	1	3	2	3	2

TABLE 17

INOCULATIONS OF HEALTHY BEET SEEDLINGS WITH CURLY TOP BY MEANS OF DIFFERENT NUMBERS OF PREVIOUSLY NONINFECTIVE MALE LEAFHOPPERS OR NYMPHS EXPOSED FOR VARYING PERIODS TO DILUTED VIRUS EXTRACTS FROM INFECTIVE BEET LEAFHOPPERS

Experiment No. 1

| Feeding period, hours | Number of insects exposed to each beet | Dilutions of virus extract from infective leafhoppers | | | | | | | |
| | | 1:100 | | 1:1,000 | | 1:5,000 | | 1:10,000 | |
		Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected
2	1	5	0	5	0	5	0	5	0
	5	1	0	1	0	1	0	1	0
	10	1	0	1	0	1	0	1	0
	20	1	0	1	0	1	0	1	0
2	1	5	0	5	0	5	0	5	0
	5	1	0	1	0	1	0	1	0
	10	1	1	1	0	1	0	1	0
	20	1	0	1	0	1	0	1	0
4	1	5	1	5	0	5	0	5	0
	5	1	1	1	0	1	0	1	0
	10	1	1	1	0	1	0	1	0
	20	1	1	1	0	1	0	1	0
4	1	5	1	5	0	5	0	5	0
	5	1	0	1	0	1	0	1	0
	10	1	1	1	1	1	0	1	0
	20	1	0	1	0	1	0	1	0
8	1	5	0	5	0	5	0	5	0
	5	1	1	1	0	1	0	1	0
	10	1	0	1	0	1	0	1	0
	20	1	0	1	0	1	0	1	0
8	1	5	1	5	0	5	1	5	0
	5	1	0	1	1	1	0	1	0
	10	1	0	1	0	1	0	1	0
	20	1	1	1	1	1	0	1	1

in the summary in this table, shows that better results were obtained with a 5 per cent solution of beet sugar and with sterile distilled water than with equal parts of steam-extracted beet-root juice and a 5 per cent solution of beet sugar.

Mass Inoculation. — An experiment was conducted to determine whether the time of exposure of varying numbers of leafhoppers to the virus extract from infective beet leafhoppers was a factor in curly-top transmission. Groups of 1, 5, 10, and 20 previously noninfective male leafhoppers were exposed to the feeding solution for periods of 2, 4, and 8 hours, and then each group was transferred to a healthy beet seedling. Various dilutions were used to determine whether single insects were able to transmit curly top by exposure to high dilutions of the virus extract from infective beet leafhoppers. Sterile distilled water was used as a diluent. The results with different dilutions in experiment No. 1 are indicated in table 17.

TABLE 17—*(Concluded)*

Experiment No. 2

Feeding period, hours	Number of insects exposed to each beet	Dilutions of virus extract from infective leafhoppers									
		1:100		1:200		1:300		1:400		1:500	
		Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected
2	1	5	0	5	0	5	0	5	0	5	0
	5	1	0	1	0	1	0	1	0	1	0
	10	1	0	1	0	1	0	1	0	1	0
	20	1	0	1	0	1	0	1	0	1	0
4	1	5	0	5	0	5	0	5	0	5	0
	5	1	0	1	0	1	0	1	0	1	1
	10	1	0	1	0	1	0	1	0	1	0
	20	1	1	1	0	1	0	1	0	1	1
6	1	5	0	5	0	5	0	5	0	5	0
	5	1	0	1	0	1	0	1	0	1	0
	10	1	0	1	0	1	0	1	0	1	1
	20	1	0	1	0	1	0	1	0	1	
6	1	5	0	5	2	5	0	5	0	5	1
	5	1	0	1	0	1	0	1	0	1	0
	10	1	0	1	0	1	0	1	0	1	0
	20	1	0	1	0	1	0	1	0	1	0
18	1	5	0	5	0	5	0	5	0	5	0
	5	1	0	1	0	1	0	1	0	1	0
	10	1	0	1	0	1	0	1	0	1	0
	20	1	1	1	0	1	0	1	0	1	0
18	1	5	0	5	0	5	0	5	0	5	0
	5	1	0	1	0	1	0	1	0	1	0
	10	1	0	1	0	1	0	1	0	1	0
	20	1	0	1	0	1	0	1	0	1	0

Since the period of exposure on unfiltered virus extracts was about 6 hours and on filtrates about 18 hours in the experiments reported in this paper, additional tests were made with groups of 1, 5, 10, and 20 male leafhoppers or nymphs exposed to various dilutions of the virus extract from infective beet leafhoppers for periods of 2, 4, 6, and 18 hours, as indicated in experiment No. 2, table 17. The dilutions were made at intervals of 100 from 1:100 to 1:500.

In experiment No. 1, table 17, 3 infections were obtained with single insects exposed to a dilution of 1:100 for periods of 4 or 8 hours, and 1 infection with a dilution of 1:5,000 for 8 hours. Two infections were obtained with groups of 5 insects exposed to a dilution of 1:100 and 1 infection with a dilution of 1:1,000 for 8 hours. Groups of 10 leafhoppers produced 3 infections after an exposure to a dilution of 1:100 for periods of 2 or 4 hours and 1 infection after an exposure to a dilution of 1:1,000 for 4 hours. Two infections with 20 leafhoppers were produced after an exposure to a dilution of 1:100 for 4 or 8 hours and 2 infections with a dilution of 1:1,000 and 1:10,000 for 8 hours.

In experiment 2, table 17, 2 infections were obtained with single insects exposed to a dilution of 1:200 for 6 hours, and 1 infection with a

dilution of 1:500 for 6 hours. One infection was obtained with each group of 5 and 10 insects exposed to a dilution of 1:500 for periods of 4 and 6 hours respectively. Groups of 20 leafhoppers produced 2 infections after exposures to dilutions of 1:100 and 1:500 for 4 hours and 1 infection after an exposure to a dilution of 1:100 for 18 hours.

As shown in table 17, 104 beets were inoculated by varying numbers of insects exposed for a period of 2 hours to various dilutions of the virus extract from infective beet leafhoppers, but only 1 beet, inoculated by 10 males exposed to a dilution of 1:100, became infected. Better results were obtained with groups of 1, 5, 10, and 20 insects exposed for a period of 4, 6, or 8 hours to various dilutions of the virus extract from infective leafhoppers. Eighty beets were inoculated by varying numbers of insects exposed for a period of 18 hours to various dilutions of the virus extract from infective beet leafhoppers, but only 1 beet, inoculated by 20 males exposed to a dilution of 1:100, became infected. During high temperatures the leafhoppers feed continuously and rarely withdraw their mouth parts from the feeding solution. During the night with a lowering of the temperature the insects do not feed as often, and this may explain the small number of infections which were obtained during a feeding period of 18 hours. The insects were fed during the afternoon and night and transferred to healthy beets during the next morning.

The infections with groups of 1, 5, 10, and 20 leafhoppers in the two experiments were as follows: 270 insects tested singly 2.6 per cent; 54 groups of 5 insects or a total of 270, 7.4 per cent; 54 groups of 10 insects or a total of 540, 9.3 per cent; and 54 groups of 20 insects or a total of 1,080, 12.96 per cent.

It may be possible that small quantities of virus repeatedly inoculated by groups of leafhoppers into many parts of a beet low in resistance may multiply and produce the disease. On the other hand, it may be possible that small quantities of virus repeatedly inoculated into the beet is not sufficient to produce infection, and that the minimal infective dose must be present in the leafhopper. If this is the case then the insects tested singly should produce about the same percentage of infection as groups of insects, provided the total number of insects is the same in each test.

DILUTION OF VIRUS FROM INFECTIVE BEET LEAFHOPPERS

The tolerance to dilution of the curly-top virus was determined with the virus extract from crushed infective beet leafhoppers. One gram of infective leafhoppers, or approximately 1,000 specimens, which had completed the nymphal stages on diseased beets were ground with pul-

verized pyrex glass with a pestle in a mortar in a Schultz mechanical grinder. To each gram of crushed leafhoppers, 99 cc of equal parts of autoclaved beet-root juice and a 5 per cent beet-sugar solution was added. The mixture containing the crushed insects was centrifuged for 1 hour at 3,500 revolutions per minute. The virus extract thus obtained was used as a stock solution for dilution. Sterile distilled water was used as a diluent. The results are indicated in table 18.

Centrifuged Virus Extract.—The tolerance to dilution of the virus extract from crushed infective beet leafhoppers was 1 :24,000, as shown in table 18. The summary in table 18 shows that 1 of 6 preparations were infectious at a dilution of 1 :22,000 and 3 of 6 preparations at 1 :20,000. With a dilution of 1 :10,000, all of the 5 preparations tested were infectious, but only 7 of 42 beets were infected in 14 feeding experiments with 5 different preparations. In two additional tests not listed in table 18, dilutions were made at 1 :45,000 and 1 :50,000 but no infections were obtained with 42 beets.

Apparently a higher concentration of the virus occurred in the infective beet leafhoppers which completed the nymphal instars on diseased beets than in diseased beet roots, if we are justified in making the comparison. One gram of beet leafhoppers was crushed in 99 cc of feeding solution, and the mixture was centrifuged, throwing down the chitin and probably most of the protoplasm. Diseased beet-root juice was centrifuged, throwing down the cellulose cell walls and probably most of the protoplasm. One cc of the virus extract from infective beet leafhoppers and 1 cc of centrifuged diseased beet-root juice was used in the lower dilutions and subdilutions were made in the higher dilutions. The dilution medium for both virus extracts from infective beet leafhoppers and diseased beet-root juice was sterile distilled water. The specific gravity of the stock solutions used for dilution with the virus extracts from infective beet leafhoppers and with the diseased beet-root juice was not determined.

It may be argued that the tolerance to dilution of the curly-top virus is not comparable to the tolerance to dilution obtained by mechanical inoculation of mosaic viruses since a small amount of virus may multiply or increase within the body of the beet leafhopper. A number of scientists have suggested that a multiplication of the virus occurs within the bodies of insects which transmit virus diseases of the yellows group. This theory was based on the fact that some insects retained the infective power through life, while others lost it quickly. The literature fails to show that a single experiment has been performed to prove or disprove the theory that the virus multiplies or increases within the body of the insect.

TABLE 18

INOCULATIONS OF HEALTHY BEET SEEDLINGS WITH CURLY TOP BY MEANS OF NYMPHS FED ON DILUTED VIRUS EXTRACT FROM INFECTIVE BEET LEAFHOPPERS

Dilutions of virus extract from infective leafhoppers

Preparation No.	1:100 Inoc.	1:100 Inf.	1:200 Inoc.	1:200 Inf.	1:300 Inoc.	1:300 Inf.	1:400 Inoc.	1:400 Inf.	1:500 Inoc.	1:500 Inf.	1:600 Inoc.	1:600 Inf.	1:700 Inoc.	1:700 Inf.	1:800 Inoc.	1:800 Inf.	1:900 Inoc.	1:900 Inf.	1:1,000 Inoc.	1:1,000 Inf.
1	6	3	6	5	6	4	9	3	6	1										
2	3	2	6	6	6	4	9	7	12	7										
3	3	0					3	0	3	2										
4	3	1	3	1			3	2	3	2	6	0	9	0	6	3	6	1	12	1
5	3	2	3	2							3	0	3	2					3	3
6	6	3									3	3	3	1	3	2	3	0	6	2
7	6	0									3	0	3	1			6	0	3	0
8	3	2									6	2	6	0	6	2	6	4	6	1
9	6	2																		
Total	**39**	**15**	**18**	**14**	**12**	**8**	**24**	**12**	**24**	**12**	**21**	**5**	**24**	**4**	**15**	**7**	**21**	**5**	**30**	**7**
Percentage		*38.5*		*77.8*		*66.7*		*50.0*		*50.0*		*23.8*		*16.7*		*46.7*		*23.8*		*23.3*

Dilutions of virus extract from infective leafhoppers

Preparation No.	1:100 Inoc.	1:100 Inf.	1:1,100 Inoc.	1:1,100 Inf.	1:1,200 Inoc.	1:1,200 Inf.	1:1,300 Inoc.	1:1,300 Inf.	1:1,400 Inoc.	1:1,400 Inf.	1:1,500 Inoc.	1:1,500 Inf.	1:1,600 Inoc.	1:1,600 Inf.	1:1,700 Inoc.	1:1,700 Inf.	1:1,800 Inoc.	1:1,800 Inf.	1:1,900 Inoc.	1:1,900 Inf.
10	3	2	3	0	3	0														
11	3	0	3	1	3	0														
12	6	2	12	3	12	0														
13	6	3	6	3	6	5														
14	3	1					6	4	6	0	6	2	6	3	6	5	6	5	6	1
Total	**21**	**8**	**24**	**7**	**24**	**5**	**6**	**4**	**6**	**0**	**6**	**2**	**6**	**3**	**6**	**5**	**6**	**5**	**6**	**1**
Percentage		*38.1*		*29.2*		*20.8*		*66.7*		*0.0*		*33.3*		*50.0*		*83.3*		*83.3*		*16.7*

Dilutions of virus extract from infective leafhoppers

Preparation No.	1:100 Inoc.	1:100 Inf.	1:2,000 Inoc.	1:2,000 Inf.	1:3,000 Inoc.	1:3,000 Inf.	1:4,000 Inoc.	1:4,000 Inf.	1:5,000 Inoc.	1:5,000 Inf.	1:6,000 Inoc.	1:6,000 Inf.	1:7,000 Inoc.	1:7,000 Inf.	1:8,000 Inoc.	1:8,000 Inf.	1:9,000 Inoc.	1:9,000 Inf.	1:10,000 Inoc.	1:10,000 Inf.
15	3	2	6	3															12	2
16	3	2	3	2															12	1
17	3	2	3	1	3	0	3	0	3	1	3	3	3	0	3	0	3	1	6	2
18	3	2	3	0	6	1	6	1	6	1	3	0	3	1	3	2	3	1	6	1
19	3	0									3	0	3	1	3	0	3	0	6	1
Total	**15**	**8**	**15**	**6**	**9**	**1**	**9**	**1**	**9**	**2**	**9**	**3**	**9**	**2**	**9**	**2**	**9**	**2**	**42**	**7**
Percentage		*53.3*		*40.0*		*11.1*		*11.1*		*22.2*		*33.3*		*22.2*		*22.2*		*22.2*		*16.7*

TABLE 18—(Concluded)

Dilutions of virus extract from infective leafhoppers

Dilution	1:100	1:11,000	1:12,000	1:13,000	1:14,000	1:15,000	1:16,000	1:17,000	1:18,000	1:19,000
20	1/3	0/3	0/3	0/3	1/3	0/3	0/3	0/3	0/3	0/3
21	3/3	0/3	0/3	0/3	0/3	0/6	0/3	1/3	0/3	0/3
22	1/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
23	1/3	0/3	0/3	0/3	0/3	0/3	1/3	0/3	0/3	0/3
24	3/3	1/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
25	1/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
26	/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
27	/3	0/3	0/3	0/3	0/3	1/15	0/3	0/3	0/3	0/3
Total	12/24	1/24	0/24	0/24	1/24	1/39	1/24	1/24	0/24	0/24
Percentage	50.0	4.2	0.0	0.0	4.2	2.6	4.2	4.2	0.0	0.0

Dilutions of virus extract from infective leafhoppers

Dilution	1:100	1:20,000	1:21,000	1:22,000	1:23,000	1:24,000	1:25,000	1:30,000	1:35,000	1:40,000
28	3/9	0/6	0/3	0/3	0/3	0/3	0/6	0/6	0/6	0/6
29	3/3	1/3	0/3	1/3	0/3	0/3	0/6	0/3	0/3	0/3
30	3/3	1/3	0/3	0/3	0/3	1/3	0/6	0/3	0/3	0/3
31	3/3	1/3	0/3	0/3	0/3	0/3	0/12	0/3	0/3	0/3
32	3/3	1/6	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
33	3/3	3/6	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Total	14/24	3/27	0/18	1/18	0/18	1/18	0/42	0/24	0/24	0/24
Percentage	58.3	11.1	0.0	5.6	0.0	5.6	0.0	0.0	0.0	0.0

Results summarized according to the number of preparations tested and found infectious

Dilution	Tested	Infectious	Dilution	Tested	Infectious	Dilution	Tested	Infectious	Dilution	Tested	Infectious
1:100	9	7	1:100	5	4	1:100	5	4	1:100	6	6
1:200	4	4	1:2,000	4	3	1:11,000	4	3	1:20,000	6	3
1:300	2	2	1:3,000	2	1	1:12,000	2	1	1:21,000	6	0
1:400	4	1	1:4,000	2	0	1:13,000	2	1	1:22,000	6	1
1:500	4	3	1:5,000	2	1	1:14,000	2	1	1:23,000	6	0
1:600	5	2	1:6,000	3	1	1:15,000	3	2	1:24,000	6	1
1:700	5	3	1:7,000	3	1	1:16,000	3	1	1:25,000	6	0
1:800	3	3	1:8,000	3	1	1:17,000	3	2	1:30,000	6	0
1:900	4	2	1:9,000	3	1	1:18,000	3	2	1:35,000	6	0
1:1,000	5	4	1:10,000	5	1	1:19,000	5	5	1:40,000	6	0

TABLE 19

Thermal Death Point of Curly-Top Virus in Extracted, Centrifuged, and Filtered Diseased Beet-Root Juice

Preparation No.	Unheated control Beets inoculated	Beets infected	55° C Beets inoculated	Beets infected	60° C Beets inoculated	Beets infected	65° C Beets inoculated	Beets infected	70° C Beets inoculated	Beets infected	75° C Beets inoculated	Beets infected	80° C Beets inoculated	Beets infected	85° C Beets inoculated	Beets infected
Extracted diseased beet-root juice																
1	3	1	3	1	3	0	3	0	6	0	6	0	3	0	3	0
2	3	0	3	0	3	1	3	0	6	0	6	0	3	0	3	0
3	3	0	3	3	3	0	3	0	6	0	6	0	....	....	3	0
4	3	3	....	....	3	3	6	2	6	1	6	0	....	....	3	0
5	3	3	....	....	3	4	6	1	6	1	18	0	....	....	3	0
6	3	2	....	....	6	....	6	3	6	3	....	....	....	....	3	0
Total	18	9	9	4	18	8	27	6	36	4	48	0	9	0	18	0
Percentage		*50.0*		*44.4*		*44.4*		*22.2*		*11.1*		*0.0*		*0.0*		*0.0*
Centrifuged diseased beet-root juice																
7	3	3	9	7	9	9	....	....	....	....	9	1	9	0	3	0
8	3	3	9	9	9	4	9	6	9	1	....	....	....	....	3	0
9	3	2	9	7	9	4	....	....	12	1	9	0	....	....	3	0
10	3	2	....	....	9	6	....	....	6	0	9	0	9	0	3	0
11	3	2	....	....	....	....	9	3	....	....	....	....	9	0	3	0
12	3	3	....	....	....	....	9	8	....	....	....	....	....	....	3	0
Total	18	16	27	23	36	23	27	17	27	2	36	1	27	0	18	0
Percentage		*88.9*		*85.2*		*63.9*		*63.0*		*7.4*		*2.8*		*0.0*		*0.0*
Filtrate prepared from diseased beet-root juice																
13	3	2	....	....	....	....	9	2	9	0	9	0	3	0	6	0
14	3	3	....	....	....	....	6	6	6	1	9	0	3	0	6	0
15	3	1	....	....	....	....	6	0	6	0	6	0	3	0	3	0
16	3	2	....	....	....	....	....	....	....	....	....	....	3	0	....	....
Total	12	8	....	....	....	....	21	8	24	1	30	0	12	0	18	0
Percentage		*66.7*						*38.1*		*4.2*		*0.0*		*0.0*		*0.0*

Results summarized according to the number of preparations tested and found infectious

Source of preparation	Unheated control Tested	Infectious	55° C Tested	Infectious	60° C Tested	Infectious	65° C Tested	Infectious	70° C Tested	Infectious	75° C Tested	Infectious	80° C Tested	Infectious	85° C Tested	Infectious
Extracted root juice	6	4	3	2	5	3	6	3	6	2	6	2	3	0	6	0
Centrifuged root juice	6	6	3	3	4	4	3	3	3	2	4	1	3	0	4	0
Filtered root juice	4	4	....	....	....	....	3	2	3	1	4	0	4	0	4	0

THERMAL DEATH POINT OF VIRUS

The thermal death point of the curly-top virus was determined in extracted, centrifuged, and filtered beet-root juice. The diseased juice was poured into pyrex glass tubing sealed at one end. The average thickness of the wall in the tubing was about 0.6 mm and the inside diameter was 8 mm. Ten cc of root juice was placed in each tube and then the open end was sealed by flame. A sealed tube was approximately three-fourths filled with juice. The tubes were submerged in a water bath controlled by an electric thermostat. A clinical thermometer was put into one tube containing the root juice, and after a number of tests it was found that it required about 1 minute for the heat to penetrate the glass tubing, and bring the temperature of the contents to that of the constant-temperature water bath. The time of exposure in the water bath was 11 minutes, 1 minute being allowed for lag. In later tests, 10 cc of beet-root juice was poured in thin-walled test tubes plugged with cotton. A submersion thermometer with 0.5° C graduations was used in the water bath. The time of exposure in the water bath was 10 minutes. After exposure to the desired temperature the tubes were cooled rapidly in running tap water. Unheated controls were always used. Determinations were made only at 5° C intervals. The results are shown in table 19.

According to table 19 the curly-top virus was inactivated in 10-minute exposure by a temperature of 75° C in the extracted and filtered diseased beet-root juice and 80° C in the centrifuged root juice. The summary in table 19 shows that of 14 preparations of extracted, centrifuged, and filtered diseased beet-root juice heated at 75° C only 1 was found to be infectious. No infections were obtained with 10 preparations heated at 80° C and 16 preparations heated at 85° C. When beet-root juice was exposed to temperatures ranging from 70° to 85° C, a coagulation of the juice sometimes occurred which may have protected the virus.

The thermal death point of the curly-top virus from crushed infective beet leafhoppers was also determined, using the same methods as were used with diseased beet-root juice. The results are indicated in table 20.

It is evident from table 20 that no infections were obtained with the virus extract, centrifuged virus extract, and filtrate prepared from the virus extract of infective beet leafhoppers heated at 80° C in 10-minute exposure. The summary in table 20 shows that of 13 preparations heated at 75° C, 4 were found to be infectious.

TABLE 20

Thermal Death Point of Virus Extract, Centrifuged Virus Extract, and Filtrate Prepared from Virus Extract of Crushed Infective Beet Leafhoppers

Preparation No.	Unheated control		55° C		60° C		65° C		70° C		75° C		80° C	
	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected
Virus extract from infective leafhoppers														
1	3	0	3	0	3	1	....	....	....	....	6	0	6	0
2	3	0	6	0	6	1	....	....	....	....	6	0	6	0
3	3	1	....	....	6	5	6	3	6	6	6	0	6	0
4	3	1	9	1	....	....	6	1	6	4	6	1	....	....
5	6	1	12	0	....	....	6	0	6	0	6	0	....	....
6	9	0	9	1	9	0	9	1	9	0	9	0	9	0
Total	27	3	39	2	24	7	27	5	27	10	39	1	27	0
Percentage	....	*11.1*	....	*5.1*	....	*29.2*	....	*18.5*	....	*37.0*	....	*2.6*	....	*0.0*
Centrifuged virus extract from infective leafhoppers														
7	6	3	9	0	9	3	9	0	9	0	9	3	12	0
8	6	3	9	2	9	1	9	0	9	0	9	0	9	0
9	3	1	9	3	9	0	9	2	9	3	9	1	9	0
Total	15	7	27	5	27	4	27	2	27	3	27	4	30	0
Percentage	....	*46.7*	....	*18.5*	....	*14.8*	....	*7.4*	....	*11.1*	....	*14.8*	....	*0.0*
Filtrate prepared from virus extract of infective leafhoppers														
10	3	3	9	3	9	0	....	....	....	....	....	....	....	....
11	3	0	....	....	....	....	6	1	6	0	6	0	....	....
12	3	0	....	....	6	2	6	0	6	0	6	0	6	0
13	3	2	....	....	....	....	6	1	6	0	6	0	6	0
14	3	2	....	....	....	....	....	....	....	....	12	1	6	0
Total	15	7	9	3	15	2	18	2	18	0	30	1	18	0
Percentage	....	*46.7*	....	*33.3*	....	*13.3*	....	*11.1*	....	*0.0*	....	*3.3*	....	*0.0*

Results summarized according to number of preparations tested and found infectious

Type of preparation	Unheated control		55° C		60° C		65° C		70° C		75° C		80° C	
	Tested	Infectious	Tested	Infectious	Tested	Infectious	Tested	Infectious	Tested	Infectious	Tested	Infectious	Tested	Infectious
Extracted	6	3	5	2	4	3	4	3	4	2	6	1	4	0
Centrifuged	3	3	3	2	3	2	3	1	3	1	3	2	3	0
Filtered	5	3	1	1	2	1	3	2	3	1	4	1	3	0

EFFECT OF FREEZING BEET-ROOT EXTRACT ON VIRUS

The filtrate prepared from undiluted diseased beet-root juice was kept in cold storage at a temperature of 0° C and –18° C. Freezing did not inactivate the curly-top virus. The number of infections obtained by exposing previously noninfective nymphs to the filtrate weekly for a period of 8 weeks and then transferring the insects to 3 healthy beet seedlings in each test is shown in table 21.

TABLE 21

INOCULATIONS OF HEALTHY BEET SEEDLINGS BY MEANS
OF NYMPHS FED ON FILTRATE PREPARED FROM DIS-
EASED BEET-ROOT JUICE KEPT IN COLD STORAGE

| Age of filtrate, weeks | Temperature in cold storage | | | |
| | 0° C | | –18° C | |
	Beets inoculated	Beets infected	Beets inoculated	Beets infected
1	3	2	3	0
2	3	3	3	3
3	3	2	3	2
4	3	1	3	3
5	3	1	3	3
6	3	1	3	1
7	3	2	3	2
8	3	0	3	2

In another test freezing did not cause an inactivation of the virus at a temperature of –18° C at the end of 11 months and 8 days.

A COMPARISON OF SOME PROPERTIES OF CERTAIN MOSAIC VIRUSES WITH THOSE OF THE CURLY-TOP VIRUS

It is not the purpose of this discussion to review all of the literature on the properties of mosaic viruses but simply to compare certain properties of mosaic viruses with the curly-top viruses of the yellows group.

Aging of Viruses in Extracted Juices.—There is a considerable difference in the longevity of various mosaic viruses in juices extracted from diseased plants. Tobacco mosaic virus has been found by all scientists to remain infectious for a considerable period after extraction from diseased plants, as indicated in table 22.

TABLE 22

COMPARISON OF SOME PROPERTIES OF VIRUSES OF MOSAIC GROUP WITH THOSE OF CURLY TOP OF YELLOWS GROUP

Virus	Aging of viruses in extracted juice*	Tolerance to dilution	Thermal death point, 10-minute exposure	Filterability
Mosaic viruses				
Tobacco mosaic (tobacco virus 1)	+3 yrs.[63], +2 yrs.[29], +4 or 5 mos., −15 mos.[1]	1:1,000,000[2,47,32]	93° C 5 minutes[3], 90° C[3], 90° C[29], 95° C[32]	Filterable[1,4,5,12,13,26,27,94]
Tomato mosaic identical with tobacco mosaic	+138 days[63]	1:10,000[63]	85° to 90° C[63], 88° C[6]	Filterable[6,63]
Yellow tobacco mosaic (tobacco virus 6)	+3 mos.[29]	1:1,000,000[47]	90° C[29]	
Tomato mosaic identical with yellow tobacco mosaic		1:10,000[56]	90° C[56]	
Speckled tobacco mosaic (tobacco virus 2)	+3 mos.[29]		90° C[29]	
Medium tobacco mosaic (tobacco virus 7)	+3 mos.[29]		90° C[29]	
Tomato-stem necrosis (tobacco virus 9)	+3 mos.[29]		90° C[29]	
Bleaching mosaic (tobacco virus 8)	−3 days[29]		75° C[29]	
Ringspot of tobacco (tobacco virus 5)	−14 days[29], −6 days[29]	1:10,000[47]	70° C[29,32]	Not filterable[45]
Mild mosaic (tobacco virus 3)	−6 days[29]		60° C[29]	

TABLE 22—(Concluded)

Virus	Aging of viruses in extracted juice*	Tolerance to dilution	Thermal death point, 10-minute exposure	Filterability
Mosaic viruses				
Spot necrosis 2 viruses	−14 days[19]	1:10,000[47]	70° C[29]	
Spot-necrosis form	−20 days[32]		60° C[32]	
Potato rugose mosaic[33]	+6 hrs., −24 hrs.[30] −24 to 48 hrs.[31]	1:10[30] 1:100[31]	Close to 43° C[30] 60° to 65° C[31]	
Mottle form	+20 days[32]	1:10,000[47]	70° C[32]	
Petunia mosaic (petunia virus)				Filterable[14,15,23,63]
Cucumber mosaic (cucumber virus 1)	−3 to 5 days[15] −3 days[29], +3 days[32] −2 days[67]	1:10,000[15] 1:100,000[32]	75° C[15] 60° C[29], 70° C[32]	
Bean mosaic	−20 to 24 hrs.[19]	1:1,000[19]	44° to 56° C[19]	Not filterable[19]
Dock mosaic	+14 days[32]	1:100[24]	80° C[24]	Not filterable[24]
Crinkle mosaic of potatoes	−24 to 48 hrs.[31]	1:10[31]	43° to 45° C[31]	
Mild mosaic of potatoes	−2 to 4 hrs.[31]	1:100[31]	40° to 45° C[31]	
Leafroll mosaic of potatoes	−24 to 48 hrs.[31]	1:200[31]	70° to 75° C[31]	
Yellows virus				
Curly top of sugar beets	−72 hrs.	1:1,000	80° C.	Filterable

* The plus sign (+) indicates that infections were obtained, and the minus sign (−) shows that the virus was inactivated.

Priode[45] found that the length of time during which the virus of ringspot disease of tobacco retains its virulence in expressed juice varies inversely with the temperature at which the juice is stored. The virus held at −5° C retained its virulence over a period of 85 days during which the experiment was in progress. A sample held at 0° C lost its virulence after about 3 weeks, one held at 10° C after 12 days, and one held at 5° C after 20 days.

Holmes[25] in working on the effect of aging on the tobacco mosaic virus found that at room temperatures the virus was reduced to 2 per cent of its original strength in a month.

Doolittle[15] found that the expressed juices of mosaic plants of several species of cucurbits were never infectious for more than 3 to 5 days and in most cases had lost their virulence within 24 to 48 hours.

Fajardo[19] found that the resistance to aging of the bean mosaic virus was 20 to 24 hours.

A rapid inactivation of the curly-top virus occurred after extracted beet-root juice was exposed to the air at room temperature for a period of 40 to 48 hours.

Resistance of Virus to Drying in Plant Tissues.—It is well known that the infective principle of common tobacco mosaic is retained in dried leaves for long periods of time. Valleau and Johnson[61, 62] have shown that old natural leaf tobacco seems to carry the virus in as virulent a form as fresh tobacco, and the disease has been produced by inoculations with samples of tobacco 5, 16, 17, 18, 20, 30, and 31 years old. Infections with preparations from dried mosaic plants have been obtained by Chapman[12] after 3 years, Beijerinck[4] after 2 years, and Allard[1] after 18 months.

Doolittle[15] found that the leaves of cucumber mosaic plants when dried at room temperatures for periods of 10 days to 1 year failed to produce the disease.

Grainger and Cockerham[24] allowed dock mosaic leaves to dry in the air for a period of 21 days and obtained infections with the inoculum prepared from them.

Fajardo[19] obtained infections with bean mosaic virus from seedlings that had been allowed to dry at room temperatures for 48 hours, but no infections were secured from plants after drying 72 hours.

The curly-top virus was inactivated in the pulp of diseased beet roots slowly dried in the greenhouse for a period of 7 weeks and in the headhouse for a period of 5 and 7 weeks, and also in diseased beet roots thoroughly dried in the greenhouse.

Tolerance to Dilution.—Allard[2] showed that the virus of tobacco mosaic could be diluted to 1:1,000 without reducing virulence. He also

made a number of successful inoculations with the dilution of 1 :10,000 and 1 :1,000,000 although these higher dilutions rarely gave infections. Walker[63] obtained infections with a dilution of 1 :10,000 with the virus of tomato mosaic, which is apparently identical with tobacco mosaic.

Doolittle[15] reported similar results with cucumber mosaic. Dilutions of 1 :1,000 were potent as undiluted solutions, but while infections may result from those of 1 :10,000 they have never taken place at higher dilutions.

Samuel[47] has shown by means of dilution tests that a light rubbing with the virus in which no visible wound is produced on the leaf, is a more effective method of mechanical inoculation than scratching with a needle in the case of five viruses diluted as follows: tobacco mosaic 1 :1,000,000; yellow tobacco mosaic 1 :1,000,000; spot-necrosis of tobacco 1 :10,000; ringspot of tobacco 1 :10,000; and petunia mosaic 1 :10,000.

Johnson and Grant[32] obtained a few infections using the rubbing method of inoculation with a dilution of 1 :100,000 with the virus of cucumber mosaic.

According to Johnson[31] the following potato viruses were for the most part relatively intolerant to dilution: crinkle mosaic 1 :10; rugose mosaic 1 :100; mild mosaic 1 :100; and leafroll mosaic 1 :200.

Grainger and Cockerham[24] found that the tolerance to dilution of the virus of dock mosaic was 1 :100.

Fajardo[19] obtained a low percentage of infections with the bean mosaic virus with a dilution of 1 :1,000.

The tolerance to dilution of the curly-top virus in beet-root juice was 1 :1,000, corresponding to the dilution magnitude of the bean-mosaic virus.

Thermal Death Point.—The thermal death points of mosaic viruses vary from 40°–45° C in mild mosaic of potatoes to 90°–95° C in tobacco mosaic in 10-minute exposure, as indicated in table 22. Exposure of the virus of tobacco mosaic to 80° C as long as 20 days did not completely destroy it, but 83°–84° C for 24 hours destroyed it.[42]

McKinney[39] found that the thermal death point was lowered approximately 6° C when juice of mosaic tobacco plants was diluted 1 :100 in 10 minutes' exposure. According to table 22 the virus of dock mosaic was inactivated when the extract was heated at 80° C for 10 minutes; this corresponds with the thermal death point of the curly-top virus.

Effect of Freezing on Virus.—Allard[3] demonstrated that fresh sap extracted from tobacco mosaic plants frozen in liquid air at a temperature of –180° C for 15 minutes at –12° C for 4 hours, and exposed out-of-doors during the entire winter and allowed to freeze and thaw repeatedly still retained its infectious properties.

Holmes[25] found that tobacco mosaic virus stored below the freezing point lost 85 per cent of its original strength after 1½ months.

Brewer, *et al.*[6] found that purified virus suspensions of tomato mosaic stored in a refrigerator at 2.22–4.44° C proved to be infectious after 6 to 20 months.

Fajardo[19] found that freezing undiluted, freshly expressed juice of bean mosaic plants at temperatures of −7° to −10° C did not affect the viability of the virus.

Doolittle[15] reported that low temperatures (specific data not given) have only a slight effect in prolonging the power of infection of the cucumber mosaic virus.

The filtrate prepared from undiluted diseased beet-root juice was kept in cold storage at −18° C. Freezing at this temperature did not inactivate the curly-top virus after 11 months and 8 days.

Filterability.—It has been shown by Iwanowski,[26, 27] Beijerinck,[4, 5] Konig,[34] Allard,[1] Clinton,[13] and Chapman[12] that the virus of tobacco mosaic is capable of passing through Berkefeld or Chamberland filters, but Iwanowski[27] found that only the first portion of the Chamberland filtrate was infectious. According to Allard[3] "the infective principle of tobacco mosaic is retained by the Livingston atmometer porous cup used as a filter, and also by powdered talc;" but Duggar and Karrer[16] readily obtained infections with infective juice passed through a Livingston spherical atmometer cup with pores noticeably finer than the average of these cups. Chapman[12] found that the juice of tobacco mosaic was still infectious after passing through a fine Berkefeld (W) candle and a Kitasata filter.

Walker[63] found that the virus of tomato mosaic, which is apparently identical with tobacco mosaic, is capable of passing through all grades of Berkefeld filters with little loss of infective power, but filtrates from the Chamberland filters gave a low percentage of infection.

Brewer, *et al.*[6] found that purified virus suspensions of tomato mosaic were still active after passage through Pasteur-Chamberland F filters and 1½ per cent Schleicher and Shüll collodion filters, but lost their virulence after passage through an atmometer cylinder, a Pasteur-Chamberland B filter, Schleicher and Shüll 3, 4½, 6, or 7½ per cent collodion filters, or 2, 3, or 5 per cent collodion filters precipitated from solution in equal parts of alcohol and ether. The wash water from the upper surface of used collodion filters was infectious.

Doolittle[14, 15] and Jagger[28] found that cucumber mosaic virus passes through Berkefeld filters but according to Doolittle[15] will not pass through all grades of Chamberland filters. Walker[63] found that

infections occurred after inoculation with the filtrate passed through the fine Berkefeld (W) filter but the filtrates from Chamberland filters gave no infections.

The curly-top virus passed through all grades of Berkefeld (V, N, W), Mandler (preliminary, regular, and fine) candles with reduced pressure, and Chamberland filters (L1, L3, L5, L7, L9, L11, and L13).

A comparison of some of the properties of viruses of the mosaic group with curly top of the yellows group indicates that curly top is caused by a specific virus distinct from viruses that have been similarly studied, although these properties lie within the range of the mosaic viruses. The properties of the curly-top virus so far investigated do not show any marked differences from those of many mosaic viruses.

SUMMARY

Nymphs after feeding on filtered and unfiltered juices extracted from the blades, petioles, and blades and petioles combined, of beet seedlings experimentally infected with curly top in the greenhouse, failed to transmit the virus to 214 healthy beets.

Infections were obtained with the juices from the blades, petioles, and blades and petioles combined, of diseased beet seedlings, extracted below the surface of autoclaved beet-root juice and a beet-sugar solution, or in a beet-sugar solution without the root juice. The results seem to indicate that oxidation was a factor in the inactivation of the virus in the first attempt.

Infections were obtained with the juices extracted from the blades and beet roots of large diseased beets removed from the field. No infections were obtained with the juices extracted from the petioles, or blades and petioles combined.

The virus can be more readily transmitted by previously noninfective nymphs exposed to centrifuged and supercentrifuged beet-root juice than by those exposed to similarly treated leaf juice; but no marked difference in the results was obtained with centrifuged and supercentrifuged beet-root juice.

Extracts from diseased leaves and beet roots were diluted with various diluents and then centrifuged, but again the preparations from beet roots gave the best results.

An inactivation of the virus occurred after extracted, centrifuged, and supercentrifuged diseased beet-root juice was exposed to the air at room temperature for a period of 72 hours. The longevity of the

curly-top virus in the filtrate prepared from diseased beet-root juice under aerobic conditions was 8 days. Infections were obtained with the partially anaerobic filtrate prepared from supercentrifuged diseased beet-root juice at the end of 5 weeks. In the filtrate prepared from diseased beet-root juice adjusted to pH 5.0 and pH 6.0 and kept in an anaerobic jar, the virus was recovered after 100 days, the full length of time this experiment was in progress. With the same juice adjusted to pH 3.5, the virus was apparently inactivated the first time that it was tested at the end of 7 days.

Attempts to cultivate the curly-top virus in a feeding solution under anaerobic conditions were failures.

The curly-top virus was inactivated in the pulp of diseased beet roots slowly dried in the greenhouse for a period of 5 weeks and in the headhouse for 7 weeks, also in diseased beet roots dried in the greenhouse for 7 weeks and in dried infective beet leafhoppers.

The virus was inactivated in beet-root juice diluted with centrifuged juice extracted from Alameda or Mammoth sweet-corn plants (immune to curly top) at the rates of 1:50, 1:100, and 1:200, after periods of 2, 4, and 6 hours.

There was no evidence to show that sedimentation of the virus occurred by supercentrifuging beet-root juice three times. There was no increase in the number of infections with the supercentrifuged liquid prepared from the gummy residue resuspended in distilled water. No infections were obtained after the first day with the filtrate containing a mixture of supercentrifuged liquid prepared from the gummy residue and aluminum gel.

The tolerance to dilution of centrifuged diseased beet-root juice was 1:1,000 and was obtained with 49 small beets removed from the field during the spring. The tolerance to dilution of the virus extract from infective beet leafhoppers was 1:24,000.

The thermal death point of the curly-top virus in beet-root juice and the virus extract prepared from infective beet leafhoppers was 80° C in 10-minute exposures.

Freezing filtered beet-root juice kept in cold storage at –18° C did not inactivate the curly-top virus after 11 months and 8 days.

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THE PENETRATION OF INSECTICIDAL
OILS INTO POROUS SOLIDS[1]

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INTRODUCTION

Many insects and other arthropod pests of economic importance hibernate during the cold season in cracks and cavities in the bark of trees and bushes. Since this occurs when the plants are in a dormant condition and therefore less subject to injury from insecticides, attention has long been directed to the possibility of applying toxic materials to the bark for the control of such pests while they are concentrated in a relatively small area and unable to escape. An example is afforded by the Pacific red spider, *Tetranychus pacificus* McG., so destructive to grapes in the San Joaquin Valley, which hibernates beneath the loose outside layers of bark on the stumps of grapevines.

The range of available insecticides for such use is very wide. However, solids either as water suspensions or as dusts have little chance of getting far enough into the bark. Fumigants are at a disadvantage for two reasons; first, their volatility is low during cold weather; and secondly, the respiratory activity of hibernating insects is very low (Bodine, 1923). This practically limits the choice to contact insecticides such as aqueous solutions of lime-sulfur, nicotine, etc.; coal-tar products, either straight or in water solution; and mineral or plant oils, either straight or in water emulsions. By adding other toxic materials which are soluble in one or another of the substances just mentioned a very large number of products of possible insecticidal value can be made.

Aside from the question of true toxicity of a given product to a particular insect, the practical effectiveness depends upon whether enough of the material penetrates through the bark to the places where the in-

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sects are hibernating. In case a web or other protective covering is present this also must be penetrated. The bark of any particular plant is not a homogeneous material but consists of a number of layers, the outer ones being corky and often adhering but loosely to the underlying layers. In the case of grapevine bark the outer layer is wet readily by water and by oils but the successive inner layers are wet by water with increasing difficulty, so that it is practically impossible for water to penetrate through the bark to the cambium layer. Oils wet all layers but penetration into the inner bark is considerably slower than into the outer bark.

The spread of either water or oil through any kind of bark is a very irregular process because small openings serve as capillary leads and narrow connecting bands between loosely adhering pieces slow down the spread very greatly. In view of these difficulties it seemed better to make the preliminary studies of the penetration of insecticidal oils with porous materials of uniform and duplicable nature. A grade of paper known as California bond (hereafter called "typewriter paper") and a deadening felt $\frac{1}{16}$ inch thick were used. These afforded, respectively, the condition of spreading through very thin, dense media and through thick, porous media. Before describing the experimental procedure it will be helpful to review briefly the process of penetration through a medium containing minute openings and cavities.

FACTORS CONCERNED IN CAPILLARY FLOW

When a capillary tube, open at both ends, is connected with a liquid at a distance below the surface, the liquid will enter the capillary under the influence of two forces, one gravitational and the other interfacial. The first is readily measured in terms of the hydrostatic head existing at any given point, e.g., in figure 1*A*, at point *a* the force due to gravity is measured by the height *h* multiplied by the density of the liquid. This is usually expressed in terms of force per unit area, which is pressure, and a wide variety of terms are in use such as dynes per square centimeter, millimeters of mercury, atmospheres, etc. This force may be either positive or negative as shown in figure 1*A* at point *b* where a negative force is exerted equal to the height *h'* multiplied by the density of the liquid.

The interfacial force, often called the capillary force, is more difficult to define. It is the result of a change in energy that occurs when the solid-air interface of the bore of the tube is replaced by the solid-liquid interface as the liquid advances into the capillary tube. At any

interface (i.e., boundary between two different kinds of matter), the balance between the forces of attraction and of repulsion which exists within the material on either side of the interface is no longer exact. This lack of balance results in a display of energy in some form. A very familiar example is the interface between water and air, ordinarily, but somewhat incorrectly, called the water surface. The lack of balance in this interface gives rise to the property called the surface tension of water, which has a definite magnitude at each temperature and can be measured by a variety of methods (Rideal, 1930, pages 1–25; National
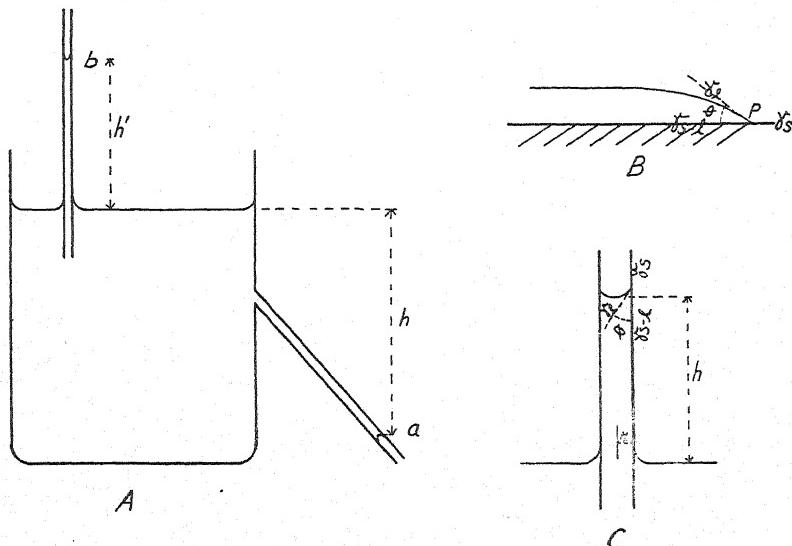
Fig. 1. *A*, The entrance of a liquid into a tube: *a*, aided by gravity; *b*, against gravity. *B*, The equilibrium condition of a liquid spreading on a solid (vertical section). *C*, The equilibrium condition of a liquid rising into a tube.

Research Council, 1928, page 435). Similarly, at any kind of interface an interfacial tension exists. Whenever a solid is concerned the interfacial tension cannot be measured by any method so far devised, and since in these cases the physical phenomena by which interfacial tensions are usually measured cannot be perceived, the more general term "interfacial energy" is used.

In the case of a liquid entering a capillary tube, it is obvious that the process results in the replacement of the original solid-air interface by a solid-liquid interface. If the original energy per unit area of interface is greater than the final energy, the difference will be available for drawing liquid into the capillary tube. While neither the initial nor final interfacial energy can be measured, the difference between them can be

readily determined. If a drop of a liquid is placed on a flat, horizontal surface of a solid the liquid will ultimately assume an equilibrium position, and if it wets the solid the final layer will be too thin for gravity to have an appreciable effect. This equilibrium position is characterized by a definite angle of contact, θ, as shown in figure 1B, which represents a vertical cross section through a drop of liquid on a solid. At point P three different interfaces meet. The interfacial energies are those of the solid-air interface (γ_s), the liquid-air interface (γ_l), and the solid-liquid interface (γ_{sl}). Since equilibrium in spreading has been reached, the energies on opposite sides of point P must be equal. Since only the horizontal component of γ_l is effective in the plane of the surface of the solid, the equation of equilibrium is:

$$\gamma_s = \gamma_{sl} + \gamma_l \cos \theta \qquad\qquad\qquad \text{I}$$

Hence the change in interfacial energy that occurs when a solid-air interface is replaced by a solid-liquid interface may be expressed:

$$\Delta\gamma = \gamma_s - \gamma_{sl} = \gamma_l \cos \theta \qquad\qquad\qquad \text{II}$$

The change in energy per unit area of interface can therefore be determined from a knowledge of the surface tension of the liquid and the angle of contact.

To apply this reasoning to calculation of capillary force it is necessary to consider a capillary tube in which a liquid of density d has risen to equilibrium as represented in figure 1C. All terms have the same meaning as in the previous figure, and as before: $\Delta\gamma = \gamma_s - \gamma_{sl} = \gamma_l \cos \theta$. The total energy available per unit length of the capillary tube is then $2\pi r \gamma_l \cos \theta$. This energy is holding up a column of liquid with a cross-sectional area of πr^2. Hence the energy per unit cross-sectional area is:

$$\epsilon = \frac{2\pi r \gamma_l \cos \theta}{\pi r^2} = \frac{2\gamma_l \cos \theta}{r} \qquad\qquad\qquad \text{III}$$

which has been called the capillary pressure, P_c (Washburn, 1921), and is the interfacial force previously mentioned which causes a liquid to enter a tube.

The relative importance of the gravitational and interfacial forces in influencing the entry of a liquid into a capillary tube may be illustrated by an example. Consider a hypothetical liquid with a density of 0.86 grams per cc and a surface tension of 28 dynes per cm, whose angle of contact on a certain solid is 5°. The gravitational force, P_g, in dynes for various heights in the tube above the liquid surface as in figure 1 is given by the product hdg in which g is the acceleration due to gravity (980 cm per sec. per sec.) and the other terms are as defined before. The inter-

facial force, P_c, in dynes per square centimeter, is given by the expression $\frac{2\gamma_1 \cos \theta}{r}$. This is worked out, for various radii of the tube in table 1. It is apparent that for small capillaries and small hydrostatic heights, which will be the conditions prevailing at the beginning of movement through capillary tubes under any ordinary conditions, the effect of gravity is negligible.

TABLE 1

RELATIVE MAGNITUDE OF GRAVITATIONAL AND
INTERFACIAL FORCES

Gravitational force		Interfacial force	
h, in cm	P_g, in dynes per square cm	r, in cm	P_c, in dynes per square cm
1	*2*	*3*	*4*
1	842.8	0.1	557.7
10	8,428.	0.01	5,577.6
100	84,280.	0.001	55,776.
....	....	0.0001	557,760.

The expression derived for the capillary pressure which causes a liquid to flow into a tube does not tell anything about the rate at which the head of the liquid column will move. In order to calculate this, certain assumptions must be made concerning the manner in which flow takes place. Poiseuille (1842, 1846) assumed that straight-line flow occurs, as distinguished from turbulent or rolling motion (Martin, 1928), that no slipping occurs at the boundary wall of the tube, and that the friction is proportional to the slip of each infinitesimally thin layer on the layer next to it. In the present case the law of Poiseuille, subject to the assumptions above, states that the volume of liquid passing a given point in a tube of fixed radius in unit time is directly proportional to the product of the pressure under which it is flowing and the fourth power of the radius of the tube and inversely proportional to the product of the viscosity of the liquid and the length of the liquid column in the tube. In symbols it may be written:

$$V = \frac{\pi r^4 P \Delta t}{8 \eta l} \qquad \qquad \text{IV}$$

in which V is the volume of liquid, P is the total effective pressure, r is the radius of the tube, Δt is the duration of flow considered, η is the viscosity of the liquid, and l is the length of tube filled. Since in a tube of radius r a volume V would occupy a length $\frac{V}{\pi r^2} = \Delta l$, in which Δl is the

distance moved in the time Δt, the above expression may be rewritten:

$$\Delta l = \frac{r^2 P \cdot \Delta t}{8 \eta l} \qquad \qquad V$$

which gives the distance that the advancing front of the column will move in the time Δt. Substituting the expression found above for the capillary pressure and neglecting the hydrostatic pressure, the following equation is obtained:

$$\Delta l = \frac{2 \gamma_1 \cos\theta}{r} \cdot \frac{r^2 \Delta t}{8 \eta l} = \frac{\gamma_1 \cos\theta \, r \Delta t}{4 \eta l} \qquad \qquad VI$$

Hence the linear rate of flow,

$$f = \frac{\Delta l}{\Delta t} = \frac{\gamma_1 \cos\theta \, r}{4 \eta l} \qquad \qquad VII$$

Since the angle of contact is zero or nearly so for most liquids on most solids this may be rewritten in its approximate form for very small values of θ, as:

$$\text{linear rate of flow, } f = \frac{\Delta l}{\Delta t} = \frac{\gamma_1 r}{4 \eta l} \qquad \qquad VIII$$

In order to change this rate equation into one involving total distance moved through, it is necessary to sum up the motions at every instant from the beginning to any time in question, i.e., to integrate the last equation above which may be written in differential form:

$$l \, dl = \frac{\gamma_1 r dt}{4 \eta} \qquad \qquad IX$$

Integrating,

$$\frac{l^2}{2} = \frac{\gamma_1 r t}{4 \eta} \text{ or } l = \left(\frac{\gamma_1 r t}{2 \eta} \right)^{1/2} \qquad \qquad X$$

The argument developed above may be applied immediately to flow through a porous medium by considering that it contains many capillaries of various sizes, lengths and arrangements, in each of which the liquid flows in accordance with the equations given above.

The term r becomes then a weighted average radius, or to be more precise it is the fourth root of the average fourth power of the radii (see equation IV), as emphasized by Stamm (1928). It will be noted that the distances to which two liquids will penetrate a given kind of porous solid in a given time vary directly as the square root of the ratio of the surface tensions and inversely as the square root of the ratio of the viscosities. For a given liquid in a given solid the equation for distance penetrated may be written, $l = K_1 t^{1/2}$. This is a particular form of the general expression, $l = K_2 t^{1/n}$, which has been found to hold for the movement of many kinds of fluids through a variety of porous solids. In

these equations $K_1, K_2,$ and n are constants, and since K_1 and K_2 are functions of both the liquid and the solid concerned these equations are of little use in classifying either kind of material.

Bell and Cameron (1906) investigated the flow of water and of aqueous solutions of several inorganic salts and dyes through filter paper, blotting paper, and soils. They found n of the above equation to be very close to 2 in all cases, whereas K_2 varied widely, as might be expected. Among recent studies is the work of Stamm (1928, 1932) who used rates of flow of liquids through thick wooden membranes in determining the number and nature of the pores in several soft woods such as red cedar and spruce. Bartell and Osterhof (1928) used the same method in a careful study of the structure of compressed carbon and silica. Ginsburg (1931) found the rate of penetration of petroleum oils into leaf tissue and into the bark of apple twigs to vary inversely as the viscosity.

As stated in the foregoing deduction, the equations derived above for the flow of liquids through porous solids hold only when spreading is far from equilibrium, and fail if it proceeds upward to a considerable distance. If this occurs the gravitational force will become significant and may be important not only by slowing down the rate of spread but also by determining the total distance the liquid will penetrate. A number of other factors may intervene to make the rate of spread less than that calculated by the equations. Thus, if oxidation of the liquid occurs, the viscosity will ordinarily increase, with consequent slowing-down of the rate of spread. Also, if the solid contains substances soluble in the liquid the viscosity may increase, e.g., the resins present in bark have this effect on light oils. If the liquid is a solution, preferential adsorption on the walls of the capillaries may occur with resultant changes in surface tension and viscosity and more or less separation of the components of the solution.

In the foregoing discussion a limit on how far a liquid will move through a capillary tube or porous solid dipping into it has been mentioned for only one condition, i.e., when the liquid rises until the capillary pressure is balanced by the hydrostatic pressure due to gravity. Theoretically there is no limit to how far movement will proceed through a tube or porous solid in a horizontal or downward direction provided there is an unlimited supply of liquid. Of course, if for any reason the viscosity increases sufficiently the motion will become infinitesimally small and if a gel is formed no motion will occur. Evaporation of a liquid from a porous solid may become equal to the rate of flow, especially if the surrounding atmosphere is moving.

The very important question of how far a given quantity of a liquid will spread through a porous solid is connected with the changes in

interfacial energy previously mentioned. If no adsorption, evaporation, or change in viscosity occurs, it is obvious that flow will continue into the various-sized spaces in accordance with theory until all the liquid has entered the solid. A second phase will then ensue during which smaller capillaries at the limit of flow will fill at the expense of larger ones previously filled. To make clear how this process is possible, it is necessary to notice once more the energy changes involved when liquid enters or leaves a capillary tube. The filling of a tube is shown in figure
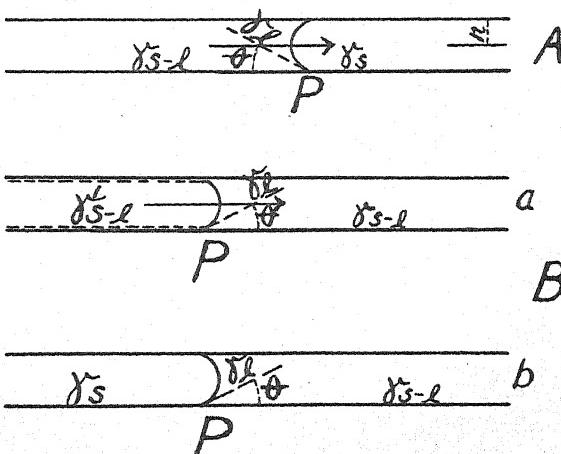
Fig. 2. *A*, Entrance of a liquid into a tube. *B*, The emptying of a tube: *a*, illustrating the thin film left on the walls when a liquid is withdrawn from a tube; *b*, final condition after the film on the walls is removed.

2*A* for which, as deduced before (equation II), the change in energy per unit area of interface is $\Delta\gamma = \gamma_s - \gamma_{sl} = \gamma_l \cos \theta$ and the capillary pressure is $\frac{2\gamma_l}{r}$ when the angle of contact is zero. Figure 2*B* illustrates the emptying of a capillary tube. Emptying is not simply the opposite of filling, for a film of liquid adsorbed on the interior surface of the tube is left behind. The process of *completely* emptying a capillary tube may be thought of as occurring in two steps: (1) removal of all liquid except a thin layer on the wall (fig. 2*B*, *a*); and (2) cleaning this adsorbed layer from the wall (fig. 2*B*, *b*). The energy changes in these two steps may be formulated as before (remembering that the signs will be opposite because the process is reversed from that of filling a tube) : $- \Delta\gamma' = \gamma'_{sl} - \gamma_{sl}$ in which $- \Delta\gamma'$ is the energy change per unit of interface for step 1, γ'_{sl} is the energy of the interface between air and the solid plus its adsorbed layer of liquid, and γ_{sl} is the energy of the solid-liquid in-

terface. And $-\Delta\gamma'' = \gamma_s - \gamma'_{s1}$, in which $-\Delta\gamma''$ is the energy change per unit of interface for step 2, γ_s is the energy of the solid-air interface, and γ'_{s1} is as before. Adding the energy changes for the two steps:

$$-(\Delta\gamma' + \Delta\gamma'') = \gamma'_{s1} - \gamma_{s1} + \gamma_s - \gamma'_{s1} = \gamma_s - \gamma_{s1} \qquad \text{XI}$$

which equals the energy change involved in *filling* the capillary tube as deduced previously (equation II). Since the energy evolved in filling a capillary tube can thus be shown to be equal to the sum of the energies consumed in emptying and cleaning the same capillary or one of equal radius, it follows that the energy required to empty a tube of all the liquid in it except the layer next to the wall is less than the energy liberated in filling another tube of equal or smaller radius, and therefore a liquid will flow from larger capillaries into smaller ones until only a thin layer of adsorbed liquid is left on the walls of the larger ones. The final condition in a porous solid containing all sizes of tubes and cavities will be for the liquid to fill completely only the tiniest capillaries and be spread over the interior surface of all larger spaces in a layer whose thickness will depend upon the properties of both the solid and the liquid.

SUBSTANCES STUDIED AND MEASUREMENT OF SURFACE TENSION, DENSITY, VISCOSITY, VOLATILITY, AND UNSULFONATABLE RESIDUE

A wide variety of spray oils was secured, most of which were the stock oils from which commercial emulsions are made. They varied considerably in the properties of unsulfonatable residue, surface tension, viscosity, and density as shown in table 2. These oils were secured from the producers in closed containers and were protected from exposure to air and light before use. In addition to the petroleum oils a number of vegetable oils, miscible oils, and chemicals that are not oils at all were also studied. These were all of technical grade since the aim was to obtain data on materials of insecticidal value rather than of high purity. For this reason the data on some of the above-mentioned properties for the materials in the last group are slightly different in a few cases from data given by other workers for pure compounds.

According to the foregoing discussion the surface tension and viscosity of a liquid affect its rate of entry into a porous solid. Reports of determination of these properties under conditions suitable for application to the results of spreading experiments are scarce. In particular, but few determinations of the surface tensions of suitable oils for use in spraying have been made, and viscosities have usually been expressed in

TABLE 2

PENETRATIVITY AND RELATED PROPERTIES OF LIQUIDS STUDIED

Substance	Unsulfonatable residue (U. R.)	Saybolt viscosity in seconds at 100° F	Absolute viscosity in poises at 25° C	Surface tension in dynes per cm at 25° C	Density, in grams per cc at 25° C	Penetrativity, in cm per sec. at 25° C	Actual penetrativity in typewriter paper at 25° C
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
Mineral oils							
1 (fly spray)	98	32	0.0125	27.0	0.796	1,080.	993.
2 (fly spray)	90	40	0.0166	27.6	0.820	832.	828.
3 (kerosene)	98	42	0.0180	27.3	0.794	760.	
4	90	50	0.0220	27.7	0.821	630.	
5	90	50	0.0235	29.4	0.853	626.	
6	99	43	0.057	29.5	0.841	259.	
7	89	44	0.062	30.0	0.855	242.	
8	70	44	0.064	30.5	0.878	238.	
9	90	55	0.107	30.5	0.859	143.	
10	90	52	0.107	30.8	0.859	144.	
11	90	56	0.140	31.4	0.877	112.	
12	92	87	0.190	31.1	0.871	81.9	equal
13	99	99	0.197	30.8	0.865	78.2	to
14	92	86	0.250	31.4	0.848	62.8	theoretical
15	65	75	0.259	32.4	0.908	62.6	
16	60	95	0.342	32.3	0.910	47.2	
17	70	100	0.369	32.7	0.914	44.4	
18	65	105	0.455	32.7	0.918	36.0	
19	70	120	0.466	32.9	0.920	35.4	
20	70	140	0.578	33.1	0.919	28.6	
21	65	200	0.941	33.2	0.918	17.7	
22	59	227	0.990	33.1	0.915	16.7	
Miscible oils							
1	....	....	0.126	25.9	0.915	103.1	69.0
2	....	....	0.410	28.6	0.909	34.9	32.0
3	....	....	0.466	32.5	0.915	34.9	15.0
Plant products							
Pine oil 1	....	....	0.0175	29.6	0.897	847.	830.
Terpinol	....	....	0.0175	29.5	0.878	843.	648.
Turpentine	....	....	0.0180	27.9	0.847	775.	664.
Pine oil 2	....	....	0.137	32.0	0.930	117.	95.0
Oleic acid	....	....	0.305	33.4	0.882	54.7	33.2
Cottonseed oil	....	....	0.559	34.2	0.910	30.6	31.0
Non-oils							
Ethyl acetate	....	....	0.0044	25.0	0.885	2,840.	1,020.
Butyl acetate	....	....	0.0070	25.7	0.860	1,840.	745.
Butanol	....	....	0.0250	25.2	0.805	504.	320.
o-toluidine	....	....	0.0345	41.0	0.992	594.	171.
Cresol (technical)	....	....	0.109	38.5	1.028	177.	61.3
m-cresol	....	....	0.118	37.6	1.026	158.	67.7
Sulfonated petroleum soap	....	....	0.780	30.6	0.924	19.6	19.4

empirical units such as Saybolt seconds, which are not directly proportional to absolute viscosities. Besides, viscosity determinations have usually been made at elevated temperatures and from the results of such experiments it is impossible to estimate even relative viscosities at ordinary temperatures where spreading ability is of practical interest. This is true because the viscosities of various oils change with temperature at different rates.

In the present work measurements have been made of the surface tensions and absolute viscosities of a considerable number of insecticidal oils and of several related substances. Values for those whose spreading powers have been studied are collected in table 2. All determinations were made in an air bath provided with a mercury regulator for temperature control, and a fan, and armholes through which instruments inside could be reached with minimum disturbance of the temperature. Observations were made through a window in one side. The temperature as indicated by a mercury thermometer never was outside the interval of $25.0° \pm 0.1°$ C. Since temperature fluctuations in an air bath are rapid, the variation in temperature of the liquids was considerably less than the interval mentioned. All liquids were put into large stoppered test tubes and left in the air bath for at least 2 hours before measurements were made on them.

Surface tensions were determined with a du Noüy Universal Tensiometer which was carefully standardized with weights (du Noüy, 1925) from a calibrated set. The liquid was poured into a 4-inch crystallizing dish for measurement. At the moment a determination was to be made the fan in the air bath was stopped to prevent motion in the surface of the liquid. An attempt was made to measure a fresh surface, since preliminary experiments showed that upon standing the surface tension decreased, particularly in the case of solutions. Each value in table 2 is the average of several closely agreeing measurements. The correction for the weight of liquid lifted above the surface by the ring was made (for the particular ring used) in accordance with the tables of Harkins and Jordan (1930). The accuracy of this correction is indicated by the fact that the surface tension of doubly distilled water was found to be 72.0 dynes per centimeter at 25.0°C (National Research Council, 1928, p. 447).

Determinations of viscosity were made by finding the time required for 2 cubic centimeters of the various liquids to flow through the capillary tube of an Ostwald viscometer. According to the equation previously deduced for capillary flow,

$$V = \frac{\pi r^4 P \, \Delta t}{8 \eta l} = \frac{K \, P \, \Delta t}{\eta} \qquad \text{IV}$$

But in the Ostwald instrument the pressure is due solely to the difference in level of the liquid in the two arms and hence is proportional to the density of the liquid. Incorporating this proportionality constant with the constant K above, the equation may be written, $V = K_1 \dfrac{d\,\Delta t}{\eta}$. Since the same volume was used in all cases the time of flow of a number of liquids of known density and viscosity was found and a graph made of $d\,\Delta t$ versus η. This would be a straight line if all the assumptions of Poiseuille previously mentioned held exactly. Actually a number of corrections are involved (Bingham and Jackson, 1917). However, these need not be calculated and they only resulted in a very slight curvature in the plot of $d\,\Delta t$ against η in the region of low viscosity. *International*

TABLE 3

RELATION BETWEEN ABSOLUTE VISCOSITY OF SUGAR
SOLUTION AND TIME OF FLOW THROUGH
OSTWALD PIPETTE

Per cent sucrose	Absolute viscosity in poises, η^*	Density in grams per cc, d^*	Time of flow in seconds, t
0	0.00894	0.9971	88.4
20	0.01710	1.0794	155.8
40	0.05206	1.1744	435.1
60	0.4402	1.2840	3,436.

* From Bingham, E. C., and R. F. Jackson. Standard substances for the calibration of viscometers. U. S. Dept. Comm. Bur. Standards Sci. Paper 298: 86-95. 1917.

Critical Tables (National Research Council, 1929) cite the data of Bingham and Jackson (with slight corrections) for the densities and absolute viscosities of water and of a number of sucrose solutions at 25.0° C. Their directions were followed in preparing the solutions, and the times of flow through the Ostwald viscometer were repeatedly determined. The results are presented in table 3. All the values in table 3 are for 25.0° C. Since the absolute viscosity of water at 20.0° C is 0.01005 poises (1.005 centipoises) the above values and all others derived by using them, when expressed in centipoises, show at once how many times more viscous than water a given liquid is.

Each of the four groups of substances included in table 2 are arranged in the order of increasing viscosity, since this property varies more widely than the other properties listed, and is very influential in determining the rate of penetration into porous solids.

Since the densities of the liquids occur in the equation for calculating viscosity, it was necessary to determine these values. They were found by weighing 50-cc volumes measured at 25.0° C and are included in

table 2. To determine the viscosity of a given liquid from its time of flow through the Ostwald viscometer, the time (in seconds) was multiplied by the density; and from the graph of $d \, \Delta t$ versus η the latter was determined. It is interesting to note that for the mineral oils the three properties, surface tension, viscosity, and density increase together fairly consistently; whereas this does not hold for the other groups. Francis and Bennett (1922) found the same relations for petroleum oils.

The volatility of certain substances was determined by finding the loss in weight when 1 gram of the liquid mixed with 9 grams of a 20–40 mesh sand in a 10–cm flat-bottomed petri dish was kept on a boiling water bath for various intervals. This is the method used by the Division of Chemistry of the California State Department of Agriculture (Elmore, 1931).

The degree of refinement of the mineral oils was found by the method used by the State Department of Agriculture (Elmore, 1931). Not all oils were tested in this laboratory; for some of them were special preparations from the research laboratories of oil companies whose characteristics had been carefully determined there. Since slight variations of the unsulfonatable residue did not enter into this work such analyses were accepted as correct.

MEASUREMENT OF RATES OF SPREADING

Straight Oils in Paper and Felt.—The experiments on rate of spreading were made by placing a few cubic centimeters of the liquid in a 1 by 8-inch test tube and hanging a strip of the solid (typewriter paper or deadening felt) from the cork in such a manner that its lower end was immersed for a short distance in the liquid, but the strip did not touch the side of the tube at any point. Two short pins stuck at right angles through the end of the strip made this easily possible. A width of ⅝ inch was convenient and was adopted as standard. Since the corks were put in firmly and not removed during the experiment the air remained practically saturated with the vapor of the liquid being studied, and loss by evaporation from the strip as the liquid rose was reduced to a minimum. The height to which the liquid had risen in the strip above the surface of the liquid in the bottom of the upright tube was measured from the outside at convenient intervals without disturbing the arrangement in any way. All experiments were repeated a number of times with close agreement. Averages are used in all calculations.

The data secured are far too voluminous to tabulate, but the behavior of several typical substances is shown in figure 3. The great differences

in rate of spread of different oils are illustrated and the effect of the nature of the solid is also shown in the case of a few oils. The equations previously deduced for the rate of flow of a liquid through a porous solid may be tested by the data secured. It will be recalled that the distance penetrated is related to the other factors by equation X, $l^2 = \dfrac{\gamma_1 r t}{2\eta}$. Hence, $\dfrac{2l^2\eta}{\gamma_1} = r\,t$ and upon plotting the left-hand term against the
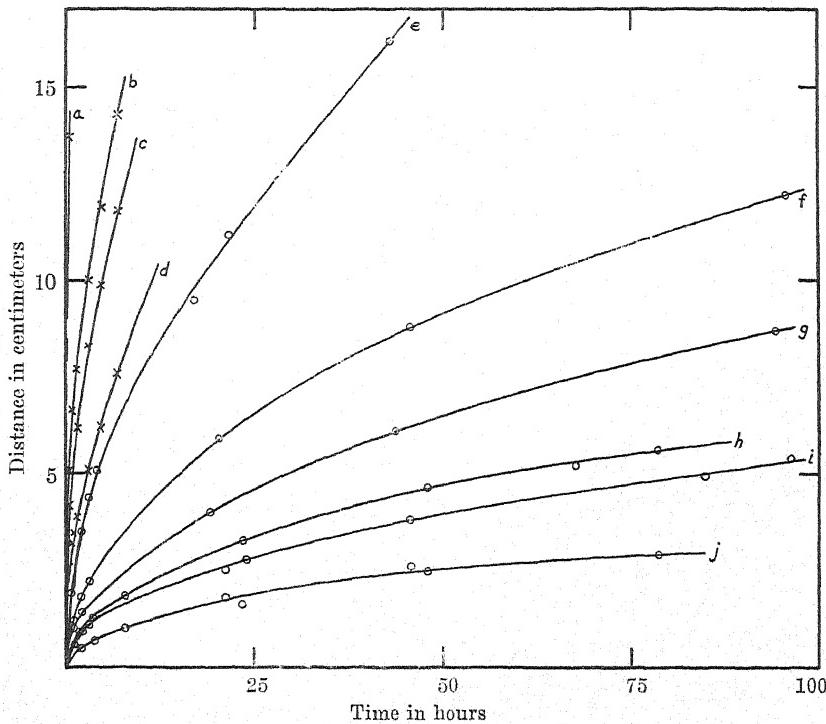
Fig. 3. Curves of distance penetrated versus time for a number of liquids in paper and in deadening felt. *a*, Kerosene in deadening felt; *b*, oil 15 in deadening felt; *c*, oil 17 in deadening felt; *d*, oil 21 in deadening felt; *e*, kerosene in paper; *f*, oil 15 + 30 per cent kerosene in paper; *g*, oil 21 + 30 per cent kerosene in paper; *h*, oil 15 in paper; *i*, oil 17 in paper; *j*, oil 21 in paper.

time, *t*, a single straight line should result for all liquids in a particular porous solid. Figure 4 shows the data obtained with the typewriter paper. On account of the piling up of points only a few can be shown on the plot, but out of 38 liquids studied 21 mineral oils (including kerosene and one fly spray), pine oil, cottonseed oil, miscible oil 2, and sulfonated petroleum soap followed the equation within the limit of experimental error up to about 25 hours, and all but the most volatile of these, e.g., the fly sprays, kerosene, and pine oil 1, followed it for about

50 hours, after which the value of the function $\frac{2l^2\eta}{\gamma_1}$ was increasingly less than the theoretical and the results were somewhat more scattering. Experiments in which the liquid was applied at the top of the strips showed that gravity was without effect and confirmed the previous assumption that the only appreciable force acting was the capillary force. Since this method of plotting rectifies the curves of figure 3, it is possible from a knowledge of the spread after one time interval to calculate it
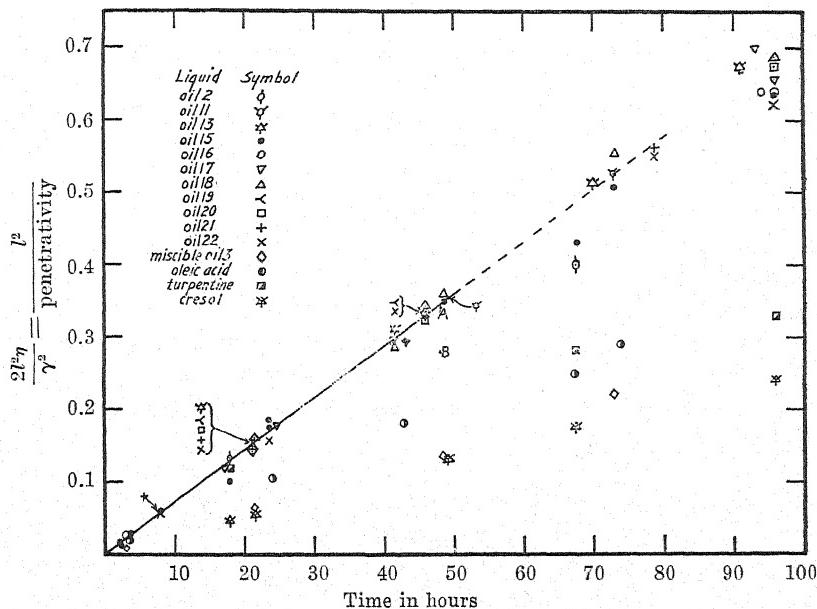
Fig. 4. Plot according to equation XII for the penetration of oils into typewriter paper.

after any other interval up to the limit at which the equation fails. By using the equation for times t_1 and t_2 it will be seen that the relation $\frac{l^2_1}{t_1} = \frac{l^2_2}{t_2}$ holds, from which the calculation follows readily. The other liquids listed in table 2 all fell below the best line drawn through the concordant results just mentioned. A few points are shown for oleic acid, turpentine, miscible oil 3, and cresol.

The fact that the majority of liquids studied obeyed the equation $\frac{2l^2\eta}{\gamma_1}$ $= r\ t$ enables a quantitative rating to be established for the spread of any desired liquid. This equation may be written $\frac{l^2}{\frac{\gamma_1}{2\eta}} = r\ t$, and if r

and t each be taken as unity, then $\frac{\gamma_1}{2\eta} = l^2$, or $\frac{\gamma_1}{2\eta}$ is numerically equal to the square of the distance to which a liquid will penetrate a tube of unit radius in unit time when the only force acting is the capillary pressure. This quantity, $\frac{\gamma_1}{2\eta}$, has been called the penetrativity (Washburn, 1921). In case the angle of contact is not zero the function $\frac{\gamma_1 \cos \theta}{2\eta}$ expresses the penetrativity. For the simple case the equation may be written:

$$\frac{l^2}{\text{penetrativity}} = r\,t \qquad\qquad \text{XII}$$

The penetrativity provides a very convenient rating of the relative rates at which liquids will enter a porous solid, and in table 2 it is given for a number of insecticidal oils and other liquids. Not only does this function give the *relative* rates of entry of liquids into a porous solid but if the graph of $\frac{l^2}{\text{penetrativity}}$ versus time has been established for any given solid, as illustrated by figure 4 for typewriter paper, then a knowledge of the surface tension and absolute viscosity of a liquid permit its penetration to be calculated quantitatively for any desired time. For example, the penetration of kerosene at 21.4 hours can be calculated as follows: From table 2 the value of the penetrativity is 760 cm per sec., and from figure 4 the value of $\frac{2l^2\eta}{\gamma_1}$ at 21.4 hours for typewriter paper is 0.162 cm per sec. Accordingly $l^2 = 123.1$ and $l = 11.1$ cm. The experimentally determined distance is 11.2 cm.

However, this use of the penetrativity is valid only for such liquids as show normal behavior. Referring to figure 4, consider a liquid which at any designated time, e.g., 48 hours, gave a point below the normal line as at B. If the penetration had been normal point A on the line would have expressed the result. From equation XII it follows that $\frac{l^2_B}{\text{penetrativity}_B} = \frac{l^2_A}{\text{penetrativity}_A}$ or $\text{penetrativity}_B = \frac{l^2_B}{l^2_A}\,\text{penetrativity}_A$. Since point A corresponds to theoretical behavior and point B to actual behavior this may be written:

$$\text{penetrativity (actual)} = \frac{l^2 \text{ (actual)}}{l^2 \text{ (theoretical)}}\,\text{penetrativity (theoretical)} \qquad \text{XIII}$$

The calculation is simplified, however, by noting that this equation may be written:

$$\text{penetrativity (actual)} = \frac{\dfrac{l^2 \text{ (actual)}}{l^2 \text{ (theoretical)}}}{\text{penetrativity (theoretical)}} = \frac{l^2 \text{ (actual)}}{\dfrac{2l^2 \text{ (theoretical)}\,\eta}{\gamma_1}}$$

$$\text{XIV}$$

or the actual penetrativity is obtained by dividing the square of the distance actually penetrated at any time by the ordinate of the line in figure 4 which corresponds to that time. For example, consider turpentine, which departs rather widely from normal behavior after 20 hours. At 24 hours the experimentally determined value of l^2 (taken from a graph of l versus t, similar to figure 3) is 114.8. The corresponding value of $\frac{2l^2\eta}{\gamma_1}$ (fig. 4) is 0.173. Hence the penetrativity (actual) is 664 cm per sec. instead of the value 775 cm per sec. as shown in column 7 of table 2. At 48 hours a similar calculation gives an actual penetrativity of 524 cm per sec. In column 8 of table 2 the actual penetrativities of those liquids which depart from normal in penetrating typewriter paper are given for 24 hours. This is, unfortunately, not a constant for any given abnormal liquid but varies somewhat as different solids are used. A further discussion of these "abnormal" liquids will be given under the heading "Total Spread of Solutions," page 76. Among liquids so far studied all mineral oils acted normally and only exceptionally volatile or reactive materials were found to be abnormal, so that it is usually possible to predict the occurrence of abnormal rate of spreading from a knowledge of the chemical properties of the substance in question. Furthermore, abnormal behavior of materials that are not mixtures was always in the direction of less-than-the-expected rates of spread.

Just as the penetrativity gives a rating of the relative rates of penetration of various liquids into a given porous solid, so does the slope of the straight line in graphs such as figure 4 give a rating of the relative rates of penetration of a given liquid into various solids. In the case of typewriter paper the slope is constant up to about 50 hours (except for the most volatile liquids). The numerical value of this slope is obtained by dividing the ordinate $\frac{2l^2\eta}{\gamma_1}$ by the abscissa (t). In figure 4 the viscosity, η, has been expressed in poises and t in hours. But to obtain r in centimeters it is necessary for t to be in seconds. Allowing for these conditions and using the data at 48 hours, $r = \dfrac{34.6}{100 \times 48 \times 3{,}600} = 2 \times 10^{-6}$ cm. While the term r has been regarded as the radius of a capillary tube or the fourth root of the average fourth power of the radii of the capillaries in a porous solid, it should not be regarded as expressing a numerical measure of the capillary size in such a material as paper, in which the capillary spaces are not all parallel but are arranged haphazard and furthermore vary through a wide range of length with spaces of unknown size and shape connecting the true capillaries. For these reasons the above value of r is regarded not as a radius of capil-

laries but rather as a measure of the relative ease with which liquids can enter such a solid.

Figure 3 also shows the l versus t curves for a few oils penetrating deadening felt, and in figure 5 the $\dfrac{2l^2\eta}{\gamma_1}$ versus t data are represented. Excellent agreement among a considerable number of oils is again shown, but the line begins to curve when the time reaches about 1 hour. Since penetration is very rapid into this material and a height of several centimeters was reached by the end of an hour, this departure from
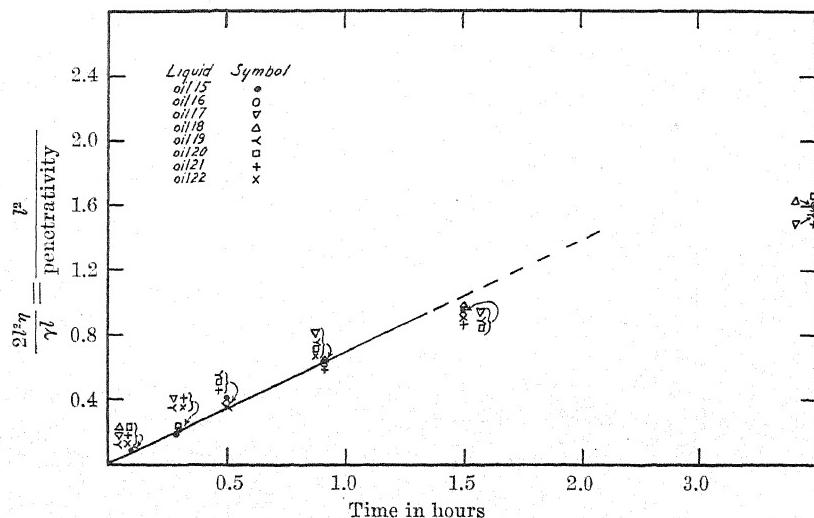
Fig. 5. Plot according to equation XII for the penetration of oils into deadening felt.

ideal conditions is to be expected and corresponds to the similar behavior shown in the case of typewriter paper after about 50 hours had elapsed. Calculating the value of r for this material in the same way as was done for the typewriter paper, the data for one hour's spread give $r = \dfrac{69}{100 \times 3,600} = 192 \times 10^{-6}$ cm. Since the corresponding term for the typewriter paper is 2×10^{-6} cm it may be seen that the deadening felt is much more readily permeable. It will be noted that since l^2 is proportional to r the following relation holds (for equal times of spread) $\dfrac{l^2 \text{ (paper)}}{r \text{ (paper)}} = \dfrac{l^2 \text{ (felt)}}{r \text{ (felt)}}$. The accuracy of this equation may be tested by the data for oil 17. For one hour's penetration into felt the value of l^2 for oil 17 is 24.8. Hence for the same interval into paper $l^2 = \dfrac{2}{192} \times$

$24.8 = 0.258$ or $l = 0.5$ cm, which agrees exactly with the experimentally determined value. By using the relation between penetration and time previously cited, namely $\dfrac{l^2_1}{t_1} = \dfrac{l^2_2}{t_2}$, the spread in paper may be calculated for any time up to about 50 hours, e.g., for 48 hours l is calculated to be 3.52 cm. Experimentally it was found to be 3.5 cm. These examples are given to show how from comparatively few data a fairly complete knowledge of the penetration of insecticidal oils into porous materials may be obtained.

Straight Oils in Bark.—Since one objective of this study on the penetration of oils was to aid in understanding what occurs when oils are applied to bark, work was undertaken on this problem. Sections of bark were removed from the trunks of various trees and grapevines. If the bark was composed of layers, as in the case of grapes, pieces of each layer were cut into strips about ⅝ inch in width and long enough to fill the test tubes. If the bark was not in layers, as in the case of oak, it was cut or sawn into sheets at various depths from the outside and strips cut as before. The rate of penetration of oils was measured exactly as for the paper and felt strips. It became apparent at once that lack of uniformity of structure in the bark leads to great variations in the rate of flow, and many strips were necessary in order to obtain an average behavior of reasonable accuracy. Also each layer of a particular bark has a different rate of penetration. Upon plotting the $\dfrac{2l^2\eta}{\gamma_1}$ versus t values for various oils penetrating each layer of a particular bark, it was shown that the heavier oils ($\eta \gtreqless 0.25$ poises) gave a fairly definite straight line for several hours' penetration, but lighter oils and particularly kerosene fell far below these lines in each case. In searching for an explanation of this behavior, microscopic observation of the surface of strips of bark as oils advanced through them showed that the tiny pieces of resin dissolved readily in kerosene but increasingly slower in heavier oils. Since solution of this material would greatly increase the viscosity, especially of very fluid liquids, it seems likely that variations from normal behavior are largely due to this cause. Using the straight lines defined by the penetration of heavier oils, tentative values of r were calculated to be:

Valley oak	23×10^{-6} cm	
Eucalyptus	34	"
Black walnut	39	"
Thompson Seedless grape	164	"

These are all for intermediate layers or sections of bark. Without citing data it may be qualitatively stated that outer layers are more readily

TABLE 4

PENETRATIVITY OF SOLUTIONS

Liquid	Distance, l in cm		Surface tension γ_l in dynes per cm at 25°C	Absolute viscosity, η at 25°C	Density, d, in gms per cc at 25°C	Penetrativity $\frac{\gamma l}{2\eta}$ in cm per sec. at 25°C	Actual penetrativity		Penetrativity (theoretical)	Penetrativity (actual)
	Paper 48 hrs.	Felt 1 hr.					Paper 48 hrs.	Felt 1 hr.	Paper 48 hrs.	Felt 1 hr.
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*
Oil 15	4.65	6.55	32.4	0.259	0.9085	62.6	62.6	62.6	1.00	1.00
Oil 15 + 5 per cent kerosene	5.5	7.25	31.9	0.212	0.902	75.4	87.5	76.2	1.16	1.01
Oil 15 + 10 per cent kerosene	6.15	7.9	31.6	0.175	0.896	90.3	109.2	90.4	1.21	1.00
Oil 15 + 20 per cent kerosene	7.5	9.05	30.9	0.117	0.886	132.0	159.0	118.0	1.20	0.895
Oil 15 + 30 per cent kerosene	8.8	10.05	30.1	0.079	0.877	190.6	223.6	146.0	1.17	0.766
Oil 15 + 5 per cent pine oil 1	5.7	7.0	32.3	0.202	0.907	80.0	94.3	71.1	1.18	0.889
Oil 15 + 10 per cent pine oil 1	6.35	7.55	32.2	0.161	0.9065	100.0	116.0	82.7	1.16	0.827
Oil 15 + 20 per cent pine oil 1	7.3	9.0	31.9	0.105	0.906	152.1	155.0	117.3	1.02	0.771
Oil 15 + 30 per cent pine oil 1	8.25	10.5	31.8	0.070	0.905	227.0	198.0	160.0	0.88	0.705

penetrated and inner layers less readily than the intermediate. The barks studied appear to lie between typewriter paper and deadening felt as regards ease of penetration. A systematic study of penetration of insecticidal oils into various kinds of bark is in progress and will be reported upon at a later time.

Solutions in Paper and Felt.—The oils previously considered have all been "straight cuts," and comprised the compounds coming over during distillation within a comparatively narrow temperature range. One way to decrease the viscosity of an oil greatly is to add a small percentage of a very fluid material such as kerosene. At the same time the surface tension is but slightly affected. Accordingly, such solutions should penetrate porous materials much more readily than the original oils. To test this hypothesis, 5, 10, 20, and 30 per cent solutions by volume of kerosene and of pine oil 1 (hereafter called the solutes) were prepared in oils 15, 17, 20, 21, and 22, and their rates of entry into the two solids, typewriter paper and deadening felt, were studied. Figure 3 gives the data for some of the solutions. The surface tensions, viscosities, and densities were determined as for the straight oils and the data for the solutions in oil 15 are included in table 4, which also gives the distances penetrated into the paper after 48 hours and the felt after 1 hour. The considerable increase in the distances penetrated runs parallel with the increases in percentage of solute and shows that small additions of such materials have very decided effects. The actual penetrativities, calculated as explained previously, are given in columns 8 and 9. When the ratio of actual to theoretical penetrativity is calculated (cols. 10 and 11) it will be noted that in the case of penetration into paper this ratio goes through a maximum at 5 to 10 per cent of solute, whereas in felt the ratio decreases with both solutes, though more rapidly with the pine-oil solutions. The circumstances that the actual penetrativity exceeds the theoretical is opposite to the case with the reactive or volatile compounds previously noted. An additional factor enters into the behavior of solutions, i.e., separation of components. This phenomenon has been studied, particularly for the case of aqueous solutions, and has usually been attributed to preferential adsorption of one component on the porous solid (Freundlich, 1930). As a result of this both the surface tension and the viscosity within the rising column are changed. To calculate the $\frac{2l^2\eta}{\gamma_l}$ versus t relation for solutions it is necessary to know (1) η versus concentration of solute for the whole range of concentration (because at the top of the ascending column the component of lower surface tension may be practically pure) and (2) concentration of the solute at each point in the ascending column. From these data the relation of η

to l could be found and the theoretical penetrativity corrected accordingly. In any particular case the same information (η to l relation) could be found experimentally by allowing the solution to rise in a roll of porous medium as was done by Fischer and Schmidmer (1893) in their study of the separation of various salts as aqueous solutions rose in filter paper. By determining the viscosity of the contents of segments of the roll at various heights above the liquid this relation of viscosity to distance risen could be found. That different solids cause separation of components to varying extents is indicated by the difference in the ratio of actual to theoretical penetrativity for paper and felt. The similar solutions of kerosene and pine oil in the other oils acted likewise.

That separation of components occurred in the present instance was proved by allowing one end of the paper strip to extend beyond the cork at the top of the test tube. The rising column of liquid extended in the course of time beyond the cork to the distance of a centimeter or so but then practically ceased to rise during a period of a few hours to a couple of days, according to the amount of solute in the solution. After this delay spreading was resumed. Obviously, the more volatile component had become concentrated at the top of the column and evaporation from the exposed strip had equaled the entry of fresh liquid until appreciable amounts of the heavier component had arrived. It is plain from the foregoing discussion that the rate of penetration into porous solids of mixtures containing components of very different characteristics does not obey the relatively simple laws that hold for straight-cut oils.

MEASUREMENT OF TOTAL SPREAD

These experiments were carried out in two ways. In the first method, called for convenience the "closed" type, a few cubic centimeters of the liquid were placed in the bottom of a test tube and a ⅝-inch strip of the solid approximately 6 inches long was fastened to a cork so that its lower end would be about a centimeter above the liquid when the cork was put into place firmly. Two pins at right angles through its lower end prevented the strip from touching the tube. A measured quantity of liquid was then allowed to run onto the lower end of the strip from a capillary pipette and the strip and cork were put into place in the test tube. The liquid at the bottom of the test tube was for the purpose of keeping the space saturated with vapor so that evaporation from the strip would be reduced to a minimum. Observations were made of the extent of spread without disturbing the arrangement. For typewriter-paper strips 0.035 cc of liquid was used and for felt strips 0.10 cc, since these volumes gave

total spreads as great as possible without exceeding the length of strip in any case.

Before discussing the results of experiments on total spread it is worth noting that so long as any portion of the liquid on the strip remained unabsorbed the rate of spread was identical with that found when the strip dipped into the liquid at the bottom of the tube, as in the arrangement for measuring rates of spread. Thus after 21.5 hours oil 15 had penetrated for a distance of 3.05 cm as compared with 3.1 cm in the other set-up. The time during which behavior thus conformed to the relation $\dfrac{l^2}{\text{penetrativity}} = r\,t$ (equation XII), was only a few hours in the case of liquids of high penetrativity and persisted for nearly two days for those of very low penetrativity. This indicates that so long as any unabsorbed oil was present the theoretical law for rate of penetration was obeyed. As before, the mineral oils comprised most of the substances obeying this law. The time at which all unabsorbed oil vanished could be determined visually with fair certainty.

The second type of experiments on total spread (called the "open" type) consisted in applying the same volume of the liquid to the lower end of strips identical with those previously described and hanging by the upper end from a horizontal bar. The strips were placed far enough apart so that slight breezes, such as occasionally swept through the laboratory, did not cause them to touch one another. This method was designed to allow full opportunity for evaporation, such as occurs whenever liquids are used as sprays in the open.

It has not been found possible to deduce a theoretical expression, in terms of experimentally determinable properties, for the total spread of a given quantity of a liquid through a solid. Accordingly, attention has been directed to determining the effect upon total spread of variations in each of several properties of the liquids used. These will be discussed as far as is practical for both "closed" and "open" conditions of spreading. The fact that several properties of spray oils vary together makes it difficult to study the effect of variation in only one at a time.

Viscosity.—It does not seem probable thas viscosity, *per se*, would have an appreciable effect upon total spread either "closed" or "open." As discussed previously, the *rates* of penetration of liquids into porous solids are inversely proportional to the viscosities of the liquids, but total spread is the distance ultimately penetrated regardless of whether reached within a few hours or after several months as actually occurred in several cases. Columns 4 and 5 of table 5 show that the time required to reach equilibrium in either "closed" or "open" penetration is dependent upon the viscosity. By thus prolonging the time required to

TABLE 5
RELATION OF OTHER PROPERTIES TO TOTAL SPREAD

Group and substance	Unsulfonatable residue (U.R.)	Absolute viscosity, η, in poises at 25°C	Duration of spread in hours — Closed	Duration of spread in hours — Open	Volatility (per cent loss in 6½ hours)	Total spread in cm — Closed	Total spread in cm — Open	Ratio of closed to open spread
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
a								
Oil 15	65	0.259	1,300–1,460	244–450	36.8	14.9	9.1	1.64
Oil 17	70	0.369	1,750–3,000	720–1,320	23.6	14.9	10.2	1.46
Oil 18	65	0.455	1,750–3,000	720–1,320	24.6	15.0	10.0	1.50
Oil 20	70	0.578	1,750–3,000	720–1,320	17.5	13.85	10.05	1.38
Oil 21	65	0.941	3,260–4,350	1,320–2,975	11.5	12.75	9.95	1.28
b								
Butyl acetate	*	0.0070	3.5–21.5	evap. <3.5	100 in ½ hr.		all evaporated	
o-toluidine	*	0.0345	67–147	evap. <3.5	100 in 1 hr.	6.65	all evaporated	
Oil 1	98	0.0125	41–67	evap. <3.5	100 in 1 hr.	8.5	2.2	3.86
Oil 3	98	0.0180	140–240	3.5–21.5	100 in 1¼ hrs.	10.9	3.2	3.41
Oil 8						16.1	4.3	3.74
Oil 13	99	0.197	1,750–2,700	675–890	36.9	18.0	11.2	1.61
c								
Oil 11	90	0.140	675–1,300	147–244	58.1	14.5	7.8	1.86
Oil 10	90	0.107	1,750–2,700	244–337	56.0	17.0	8.9	1.91
Oil 12	92	0.190	1,750–2,700	720–1,320	36.9	17.0	10.7	1.59
d								
Pine oil 1		0.0175	3.5–21.5	3.5–21.5		10.2	2.3	4.44
Terpinol		0.0175	3.5–21.5	<3.5		8.3	1.7	4.88
Turpentine		0.0180	67–147	3.5–21.5		5.3	1.3	4.08
Pine oil 2		0.137	673–1,300	3.5–21.5		9.9	1.9	5.21
Oleic acid		0.305	673–1,300	244–720		8.1	5.2	1.56
Linseed oil		0.497	140–240	147–244		5.7	4.6	1.24
Cottonseed oil		0.559	340–500	147–244		8.4	5.6	1.50

* The term "unsulfonatable residue" has no meaning for substances such as butyl acetate or o-tuluidine, but they are comparatively nonreactive and hence comparable to highly refined oils.

reach the full extent of spreading, a high viscosity increases the loss by evaporation and the extent of reaction with the air, which results in gummy substances that spread but little. This latter circumstance particularly concerns oils of low refinement. In column 7 of table 5 it will be noted that the oils of group *a* in the "closed" tests spread to the same extent within the limit of experimental error until the absolute viscosity exceeded 0.455 poises (Saybolt viscosity was 105 seconds at 100° F for that particular oil). The two oils of higher viscosity showed a marked falling-off in total spread. In the case of the highly refined substances of group *b* the most viscous one spread the farthest and the variations in spread can be explained in terms of volatility.

Volatility.—The great effect of this property upon total spread is well illustrated in table 5. Among the compounds of group *a*, all of which are but slightly volatile, the only effect was that the "open" spread of the most volatile material, oil 15, was diminished. Among the substances of group *b* this effect was very marked for both types of spread. This may seem peculiar in the case of "closed" spread in which an attempt was made to keep the space within the test tube saturated with the vapor of the liquid concerned. However, some of this vapor was adsorbed in the solid shortly after its introduction and the small surface of the liquid in the bottom of the tube prevented the maintenance of saturation thereafter. Consequently, material of high volatility evaporated from the rising column and was adsorbed above with the result that the total "closed" spread was shortened. This state of affairs was very clearly manifested with o-toluidine, which spread only 8.5 cm, although the entire portion of the strip above was colored a distinct brown. Other volatile substances did not impart a color to the upper part of the strips but a change in reflecting power was easily noted, e.g., with pine oil 1. The materials of group *c*, which are of intermediate volatility, showed the same effect of volatility upon the total spread. This effect of volatility doubtless was present in the determinations of rate of spread, but since the time was very short the error involved was entirely negligible.

If the ratio of total spread "closed" to total spread "open" is calculated for the spray oils of table 5 and plotted against the volatility, a straight line results, as may be seen by plotting column 6 versus column 9. If the volatility were expressed by the percentage evaporated after some other time than 6½ hours on the steam bath, such close agreement might not be found, but the general truth of the relation is evident.

Surface Tension and Penetrativity.—Neither surface tension nor penetrativity has a direct relation to total spread of either type. However, in a graph of total spread "closed" versus penetrativity (fig. 6)

for all materials listed in table 2, the spray oils of penetrativity between 17 and 140 cm per sec. all lie in a group having total spread between 12.2 and 18.0 cm and fairly definitely determine a line in the upper left-hand corner of the graph. All other materials of the table lie far below or to the right of this line. All substances of high volatility or reactivity gave much less total·spread than their penetrativities would indicate. Since the penetrativity involves the viscosity this graph illustrates very forcibly the result of loss in spreading power due to evaporation or reaction with the air, as discussed before. Total spread "open" showed the same effect to an even more marked extent.

Fig. 6. Relation of penetrativity to total "closed spread" in paper. Numbers correspond with numbers of oils in table 2.

Refinement.—In the case of spray oils the degree of refinement is measured by the change in volume when an oil is treated with concentrated sulfuric acid under standard conditions (Elmore, 1931), and the results are expressed in terms of unsulfonatable residue. The unsulfonatable residue (U.R.) is thus a measure of the compounds which are too inactive chemically to be affected by hot, concentrated sulfuric acid. From the standpoint of oils as spray material the U.R. is of importance both as regards ease of oxidation by the air and effect upon plants and insects (Gray and deOng, 1926; deOng, 1931). In spread through an inert porous solid, oxidation by the air is the principal factor concerned. Table 5 shows that the total spread either "closed" or "open" is influ-

enced by the degree of refinement, e.g., oils 15, 12, and 13 are closely
alike in all properties except unsulfonatable residue (which was 65, 92,
and 99 respectively) and their total spreads are 14.9, 17.0, and 18.0
cm "closed" and 9.1, 10.7, and 11.2 cm "open," respectively.

Another illustration of the same behavior is afforded by the total
spread "closed" in felt strips of a number of oils in atmospheres of air
and nitrogen. Typical of many results are those in table 6. In an inert
atmosphere no difference in total spread was found, but in air the most
reactive oil spread the least.

TABLE 6

EFFECT OF ATMOSPHERE UPON TOTAL SPREAD

Oil	Unsol-fonatable residue	Viscosity, in poises	Spread		Spread in nitrogen
			In air, in cm	In nitrogen, in cm	Spread in air
1	*2*	*3*	*4*	*5*	*6*
8	70	0.064	9.6	10.0	1.04
7	89	0.062	9.8	10.0	1.02
6	99	0.057	10.0	10.0	1.00

Effect of Amount of Liquid on Total Spread.—It might be expected
that the total spread of a highly refined oil under conditions minimizing
evaporation would be closely proportional to the volume used, but that
with volatile or reactive materials larger volumes would not spread as
far as would be indicated by the spread of small volumes because of the
longer time of spreading. To test this point various volumes of several
substances were used with paper strips under "closed" conditions with
the results shown in table 7. These results show clearly that total spread

TABLE 7

EFFECT OF VOLUME UPON TOTAL SPREAD

Material	Actual total spread "closed", in cm		Calculated for 0.35 cc from 0.020 result, in cm
	Volume		
	0.020 cc	0.035 cc	
1	*2*	*3*	*4*
Mineral oil 13	10.3	18.0	18.0
Mineral oil 3	9.3	16.1	16.3
Mineral oil 12	11.0	17.0	19.2
Mineral oil 21	10.4	13.1	18.2
Mineral oil 15	11.4	14.2	19.6
Linseed oil	5.1	5.7	8.9
Cottonseed oil	5.2	8.4	9.1
Pine oil 2	8.1	9.9	14.2
Pine oil 1	8.6	11.6	15.0

is less and less proportional to volume used as the liquid is more volatile or reactive.

Another question of considerable importance concerns the effect of adding another portion of oil to a strip in which spreading has reached equilibrium. To strips in which oils 15, 18, and 17 had completed their spread under "closed" conditions an additional 0.035 cc of each was added. The fresh oil could be seen ascending the strips and within 16 to 24 days, the time depending upon the viscosities of the oils, the limit of original spread was reached. Without pausing at this point each oil continued to spread until, after periods of about 40 to 60 days more, equilibrium was again reached. The extent of additional spread was very little, however, being 2.0, 2.2, and 1.9 cm for oils 15, 18, and 17 respectively. Experiments with other oils and with solutions containing kerosene, sulfonated petroleum soap, etc., corroborated this finding that the addition of more liquid results in but little more spread, probably because of evaporation during the long time of spreading.

Total Spread of Solutions.—The same solutions of the five oils with kerosene and with pine oil 1, that were mentioned under rates of spreading, were used to determine their total spread through paper strips. Under conditions of "closed" spreading an increase resulted, roughly proportional to the percentage of solute added. On the other hand the total "open" spread was decreased proportionally. The results may be summarized briefly by stating that the solutions containing 30 per cent kerosene gave an average increase in "closed" spread of 3.5 cm as compared with the five straight oils, whereas in "open" spread the average decrease was 1.1 cm. These are 25 per cent and 11 per cent, respectively, of the total average spreads of the straight oils under the same two conditions. With the corresponding pine-oil solutions the increase averaged 2.7 cm (20 per cent) and the decrease averaged 0.6 cm (6 per cent). Solutions of the same oils containing 30 per cent of a sulfonated petroleum soap spread less than the straight oils under both conditions, the decreases being 1.2 cm (9 per cent) "closed" and 2.1 cm (22 per cent) "open." The addition of kerosene or pine oil or sulfonated petroleum soap to the highly refined oil 13 led to a decrease with both "open" and "closed" spreads.

Similar experiments with deadening felt gave no results because the total spreads could not be determined accurately. Preliminary work with bark showed that the solvent effect of very light oils upon the bark resins therein decreased the total spreads just as it decreased the rate of spread.

DISCUSSION

The foregoing account of the entrance of a liquid into a porous solid has shown the physical necessity for the filling of pores and cavities of all sizes near the point of entry when there is an abundance of liquid. After all the liquid has entered such cavities a redistribution occurs during which all the liquid except a thin coating leaves the larger openings and enters smaller ones at the limit of spread. The whole process can be observed readily by placing a drop of oil on a piece of filter paper and viewing it through a low-power microscope. An instrument equipped with a vertical illuminator is particularly useful in watching the sequence of events.

From the data obtained on rate of spreading and total spread certain conclusions can be drawn concerning the type of oil capable of giving the greatest spread in such porous materials as bark. In the first place it should be noted that there is considerable difficulty in applying sufficient oil to completely fill the bark of such a trunk as that of a grapevine or of a tree with thick bark, and if enough oil is used to accomplish this the cost becomes excessive. Hence a material which spreads far is necessary. The characteristics which contribute to this property have been shown to be: high refinement, low viscosity, and low volatility. Increase in cost mounts rapidly with the degree of refinement, but oils of refinement intermediate between typical winter and summer grades spread well and are not very much more expensive than those of low refinement. Since the viscosities of oils having the same volatility are lower the greater the ratio of hydrogen to carbon, i.e., the more completely saturated the molecules are (Dunstan and Thole, 1918), it would be very interesting to study the relative penetration of paraffin-base and asphaltic-base oils, which differ decidedly in this ratio.

Unfortunately, petroleum oils of any origin, which have really low viscosities, e.g., kerosene, are all rather volatile and their total spreads, particularly in the open, are accordingly lessened. A compromise is necessary between the two properties, the choice in any particular case depending upon the distance through which oil must penetrate to get to the insect concerned. Another reason why oils of very low viscosity are not satisfactory for use in bark is their solvent effect on resins, which results in greatly reduced penetration unless very large amounts of oil are used.

It has been noted that if an oil spreads rapidly enough the losses by evaporation and reaction with the air are lessened. This rate of spread-

ing depends upon the penetrativity, which varies directly as the surface tension and inversely as the viscosity. Since the surface tensions of oils fall within rather narrow limits and since components of low surface tension always gather at an interface (Rideal, 1930, p. 48–58), it does not seem possible to profit by increasing this property. On the other hand, the viscosity is readily and decisively lowered by the addition of a small percentage of very fluid solutes. Since the actual penetrativity of such solutions is greater than the calculated value when the solid is difficult to enter, such solutions offer promise of being very useful. Search should be made for substances of low volatility and low viscosity which are freely soluble in oils. Cresol or cresylic soaps do not satisfy the need, for in table 2, it will be noted that the actual penetrativities of the miscible oils were considerably less than the calculated values, and data not shown in the tables indicated that both types of total spread were considerably less than were obtained with straight oils of similar properties. Vegetable oils also failed to give promise, as indicated in table 5, group *d*. It is not impossible, however, that vegetable oils with the unsaturated compounds removed might be of value in conjunction with mineral oils, especially since some have been found to be decidedly toxic (Siegler and Popenoe, 1925; Tattersfield and Gimingham, 1927).

Preliminary field trials have borne out the conclusions discussed above. Thompson Seedless (Sultanina) and Alicante grapevines were sprayed with a number of oils and mixtures during the dormant season and account was taken of the penetration through the bark and the percentage of hibernating red spiders that were killed. Very briefly summarizing this work, it may be said that very volatile materials such as oil 1 (a fly spray), kerosene, and pine oil 1 penetrated very rapidly but were apparently all evaporated within a few hours and very little kill was obtained; complete penetrations and fairly high kills were obtained with such materials as oils 12 and 15; and unsatisfactory penetrations and kills were obtained with oils of high viscosity.

The important topic of using oils as carriers for such toxic substances as naphthalene and paradichlorobenzene has not yet been studied in the present work. It is usually held that such carrying action occurs, but the separation of components noted with solutions of two liquids gives rise to doubt as to what happens when one component is a dissolved solid.

No mention has been made so far of the effect of moisture in the solid. That is not usually concerned when oils are applied straight unless a rain has come just before spraying must be done. But the penetration of oils from water emulsions is directly connected with the effect of water

upon the penetration of oil through solids. Felt strips were suspended in test tubes above a layer of water and left for one week by which time saturation with water vapor may be assumed to have been reached. A volume of oil (0.10 cc) was then placed at the bottom of the strip and the spread observed. With all six of the oils so studied the rate of penetration was less than with dry strips, and the final spread attained was also decreased. To test the ease of replacement of water by oil, or vice versa, opposite ends of strips of filter paper and of deadening felt were dipped into oil and into water contained in two beakers standing side by side and both being within a covered battery jar. The air within the jar was thus saturated with both water and oil vapor. Under such conditions the line of contact between the water and oil was very sharp and no movement in either direction could be detected in several weeks. However, if the cover was removed from the battery jar so that the water could evaporate from the strip the oil advanced steadily until it nearly reached to the water level in the opposite beaker. This experiment indicates that when an oil emulsion is sprayed upon bark, the water, being present in great excess, soaks into the bark, particularly the outer layers, and the only oil deposited is a very little upon the very outer surface. As the water then evaporates the oil penetrates into the bark. This explains why it was found quite impossible to apply enough oil emulsion to grapevines to penetrate to the hibernating red spiders.

SUMMARY

The rate of penetration of liquids into a porous solid has been shown to be controlled by a property of the liquid, the penetrativity, and by a property of the solid, the average radius of its pores. The penetrativity is a function of the surface tension and the absolute viscosity of the liquid. A standard method of studying the rate of penetration has been employed in which strips of the solid (paper, deadening felt, or bark) were dipped into oils and other liquids at the bottom of test tubes. Mineral oils were found to follow the theoretical behavior, but very volatile or reactive materials spread less rapidly than predicted. The solvent action of light oils upon the resin in bark decreased the rate of penetration.

For a wide variety of spray oils the penetrativity gave a measure of the relative rates of entry, and from graphs of spread, penetrativity, and time, the relative ease of entrance into various solids could be determined. Data on surface tension, absolute and Saybolt viscosities, density, unsulfonatable residue, volatility, and penetrativity are given,

together with details of the methods of determination. Solutions containing 5 to 10 per cent of a very fluid substance such as kerosene spread somewhat faster than was calculated from their penetrativities. This was shown to be due to separation of the components.

The total spread of known volumes of liquids through the same solids was studied by adding the liquids to strips either hung in closed test tubes or in the open air. No theoretical calculation of total spread could be made. Viscosity is important because it controls the time until maximum spreading is attained, and hence opportunity for evaporation and reaction with the air increases as the viscosity becomes greater. Volatility is of great importance especially when evaporation occurs freely. The higher the degree of refinement the greater the spread of oils, both in the open and within closed containers. Increasing the volume of oil gave spreads less than proportional to the volumes used, and the addition of more oil after spreading had ceased resulted in but little additional spread. Addition to the oils of fluid materials such as kerosene increased the total spread when evaporation was prevented but decreased it in the open.

The rate of spread and the total spread were decreased by adding moisture to the strips. The difficulty of applying enough oil in aqueous emulsions is discussed. Preliminary field experiments bore out the need for using oils of fairly high refinement, low viscosity, and low volatility in as far as these properties are mutually compatible.

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BACTERIAL CANKER OF STONE-FRUIT TREES IN CALIFORNIA[1,2]

EDWARD E. WILSON[3]

INTRODUCTION

The gummosis phenomenon in trees of the genus *Prunus* has been much investigated since the early nineteenth century. Although many studies were primarily concerned with the origin and composition of the gum itself, some attention was paid to cause. In general, most investigators regarded climatic and soil factors as the cause of the gummosis. Apparently Brzezinski,[10] working at Krakow in 1902, first attributed to bacteria a certain gumming cankerous disease of apricots, plums, and cherries. He claimed to have demonstrated the pathogenicity of these bacteria to their respective hosts, but neither named nor described them.

In 1905, Aderhold and Ruhland[1,2,3] found a bacterial canker disease, caused by *Pseudomonas spongiosa,* producing severe damage to cherries in Germany. Their work, which received wider recognition than that of Brzezinski, served to focus the attention of plant pathologists on the role of bacteria in producing gummosis of limbs and trunks of the stone-fruit trees; all types of this gummosis had heretofore been thought to result from purely physiological causes.

Griffin's work,[13] in 1911, definitely established the bacterial origin of a gummosis disease of cherry limbs in Oregon and also showed that the bacterium *(Pseudomonas cerasus)*[4] produced a blighting of dormant buds. Barss,[5,6,7] who followed Griffin in this work, demonstrated

[4] The possessive, *cerasi,* is preferable to *cerasus.*

that this same bacterium produced cankers on limbs of practically all
the stone fruits. These two workers introduced the terms "bacterial
gummosis" or "bacterial canker" to distinguish their disease from gum-
mosis resulting from other causes.

INVESTIGATIONS OF A GUMMOSIS AND A "SOUR-SAP" DISEASE IN CALIFORNIA

In California, in 1916, Barrett[4] discovered a severe cankerous con-
dition of apricot trees, with all the symptoms described by Griffin in
Oregon. After this work the disease in California was regarded as the
"bacterial gummosis."

For a number of years, a severe cankerous condition of plum trees
has occurred annually in the foothills of the Sierra Nevada Mountains,
causing especially great damage in the fresh-fruit district of Placer
County. Goldsworthy and Smith[15] have reported studies on the dis-
ease under the name of "sour-sap." Although they found symptoms
differing somewhat from those of "bacterial gummosis," no one species
of bacteria appeared to be constantly associated with the trouble; any of
several types could produce the "sour-sap" symptoms. The data pre-
sented herein are based upon a comparison of "sour-sap," as described
by Goldsworthy and Smith, with the more common gummosis disease.

INJURIES FROM OTHER CAUSES SOMETIMES CONFUSED WITH THE BACTERIAL DISEASE

Before the symptoms of "sour-sap" and gummosis are detailed, it
should be emphasized that the term *sour-sap* here designates the symp-
toms described by Goldsworthy and Smith.[15] The term as used by
some other workers refers to troubles unrelated to the disease now under
consideration. Thus Cockayne[12] and Waters[24, 25] mentioned a sour-
sap of pears in New Zealand as caused by excessive soil moisture; Miss
Willis[26] applied the term to a root trouble of prunes in Washington,
which she attributed to drought injury; Miss Phillips,[17] in California,
mentioned a sour-sap disease of apricots that was later shown to result
from *Verticillium alboatrum;*[18] and Birmingham[8] has recently re-
ported a sour-sap of cherry tree in New South Wales, which he attrib-
utes to extreme variations in soil moisture.

With the exception of Phillips, all these workers had reference to a
browned and fermented condition, particularly within the bark of
trunks and within the cortex of roots and crowns of the trees. The term
sour-sap has therefore been applied to certain symptoms but not, in
general, to any particular disease.
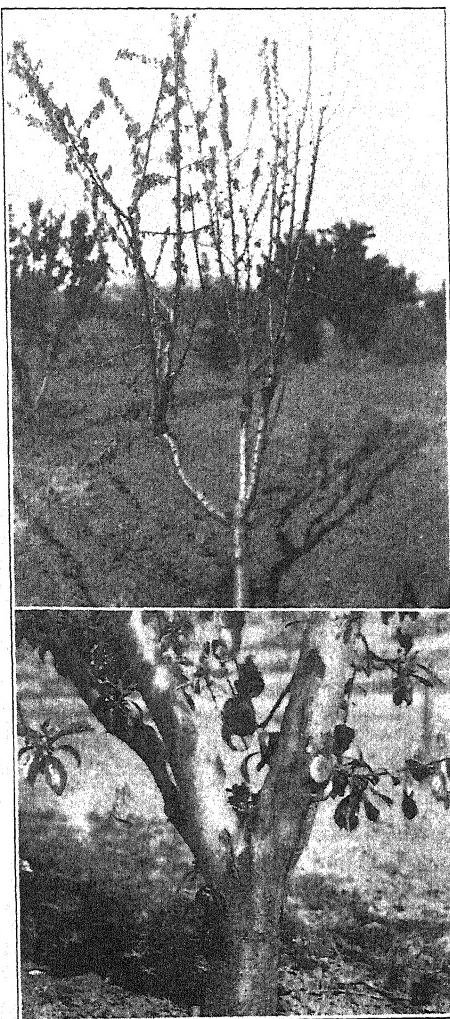
Fig. 1.—Upper, young plum tree which died from the sour-sap type of disease. Limbs on right side of tree died at the time they began to leaf-out; those on left side died after a good many leaves had been produced. Lower, Grand Duke plum with large diseased area on trunk and first scaffold branches. Note the absence of gum.

Although Goldsworthy and Smith[15] attributed most of the trouble in Placer County to the bacterial form of sour-sap, they recognized the presence of a second trouble, resulting—as they thought—from adverse soil moisture conditions. The present investigation soon showed that the death of trees in this region was not caused in every case by the bacterial disease. Trees in low-lying areas in the orchard often died in greater numbers than those in better locations, and in these cases the roots and crowns died first, a symptom not generally associated with the bacterial form of trouble. Isolations from such affected crowns and roots failed to show any consistent bacterial or fungal flora. Studies were therefore instituted for the purpose of answering two questions: (1) Does soil moisture play a part in producing this type of trouble; and (2) does soil moisture influence the bacterial disease? This work has been done in coöperation with Dr. A. H. Hendrickson of the Pomology Division, who takes full responsibility for the soil moisture phase of the problem. The results of the studies are reserved for a future publication.

SYMPTOMS OF GUMMOSIS AND SOUR-SAP COMPARED

The disease known as sour-sap in the Placer County fruit district is characterized by the failure of entire trees or portions of trees to produce leaves in the spring (fig. 1A). Some may put out foliage, only to die shortly thereafter. The bark of limbs or trunks is girdled by necrotic areas, the tissue of which is dull brown, moist, and sour-smelling. After a tree is dead one can seldom determine whether death was preceded by a girdling of the trunk or by a general involvement of the entire above-ground system, inasmuch as the bark of even the smallest limbs turns brown. Studies of numerous cases, however, have revealed that death of the entire tree follows only if the trunk has been previously girdled by the diseased area (fig. 1B).

Cankers start as small, brown to reddish-brown spots in the outer bark (fig. 2A, B). When conditions are favorable, these spots enlarge by means of small, water-soaked streaks, which during the fall and winter extend up and down the branch. Sometime during early spring a brownish discoloration begins to appear in the tissue between the streaks. This dying of the bark proceeds rapidly at about the time the buds begin to open; and by the time the leaves appear, the area that earlier manifested the disease only by the presence of the streaks now becomes uniformly brown and moist (fig. 2C). As a rule little, if any, gum is exuded from the affected tissues; but a watery material may

flow from cracks in the bark and cover the limbs. The absence of gum is particularly noticeable in the case of plums. A more detailed description of the cankers will follow later.

One rather striking and unique feature of the disease is its remaining

Fig. 2.—Sour-sap cankers. *A, B,* young cankers (indicated by arrows), visible from the exterior only by slight sinking or cracking of the periderm. *C,* periderm removed from an area beyond the necrotic center of a canker (visible in the upper right-hand corner). This area is beginning to turn uniformly brown. *D,* a diffuse canker, composed of a small necrotic center (indicated by arrow) surrounded by a wide zone of loosely connected, reddish brown streaks. The only exterior signs of this canker were a slight sinking and cracking of the periderm.

aboveground. The writer has never found a proved case in the roots. It generally stops at the ground level or slightly below.

The more common gummosis type of disease has been generally regarded as differing from the sour-sap in the presence of abundant gum and in better defined, deeper cankers. A detailed comparison of symptoms, however, has failed to show any differences that remained constant throughout the year. Such differences as appear at certain times

are those of degree. Thus, though the cankers of the sour-sap type do not usually exude gum, they have been found to do so at certain times and under certain conditions. Strong evidence, related both to the feature of gum formation and to the character of the cankers, indicates that factors other than the causal organism may influence the entire symptomatic picture. A careful comparison of cankers suggests that in European varieties of plums *(Prunus domestica)* most cankers are well-defined areas, while on trees of Japanese varieties *(Prunus salicina)* they are generally ill-defined. Gum flow is likely to be more profuse in the Japanese varieties than in European. Peaches, cherries, and apricots, furthermore, exude gum more readily than do plums. All these facts have tended to discount the importance of symptoms that at first appeared to differentiate the gummosis and sour-sap types of cankers.

Although these studies showed the great similarity between cankers that were first regarded as of two symptomatic types, dependence for more conclusive evidence was naturally placed upon isolations and inoculations. Before taking up these results, however, one should consider some other symptoms whose relation to the canker phase will be explained later.

Dormant-Bud Blight.—Both Griffin[13] and Barss[5, 6, 7] showed the blighting of dormant buds to be a phase of bacterial gummosis, common on cherries during the years when they were working with the problem.

The blighting of dormant buds by bacteria, though by no means rare in California, has been markedly less prevalent than the limb-canker phase, having been noted only on cherries, apricots, and one variety of peach, the Phillips Cling. It is recognized by failure of the buds to start growth in the spring. The affected buds are darker in color than healthy ones and are subtended on the branch by a small canker (fig. 3*A*), which generally exudes gum—sometimes in such abundance as to cover the bud. In California the fungus *Coryneum beijerinckii* Oud. produces on apricot a very similar trouble, which may be identified by the characteristic spores of the fungus appearing between the bud scales.

Blossom Blight.—The only case of blossom blight observed was found on apricot twigs sent from the Santa Clara Valley. Although the disease was thought at first to be brown rot, caused by *Sclerotinia fructicola,* subsequent isolations proved it to be a bacterial trouble. Close study indicated that the bacteria had entered through the base of the bud rather than through the blossoms. Probably, therefore, this was not strictly a blossom blight, but merely a girdling of the pedicels, after the blossoms had separated in the bud, by a lesion that had been established before the blossoms opened.

Green-Shoot Blight.—Up to the time the writer published his comparison[27] of *Pseudomonas prunicola* with the common gummosis bacterium of California, no case of the disease on green shoots of *Prunus* had been found. This publication stated: "Even though natural infec-
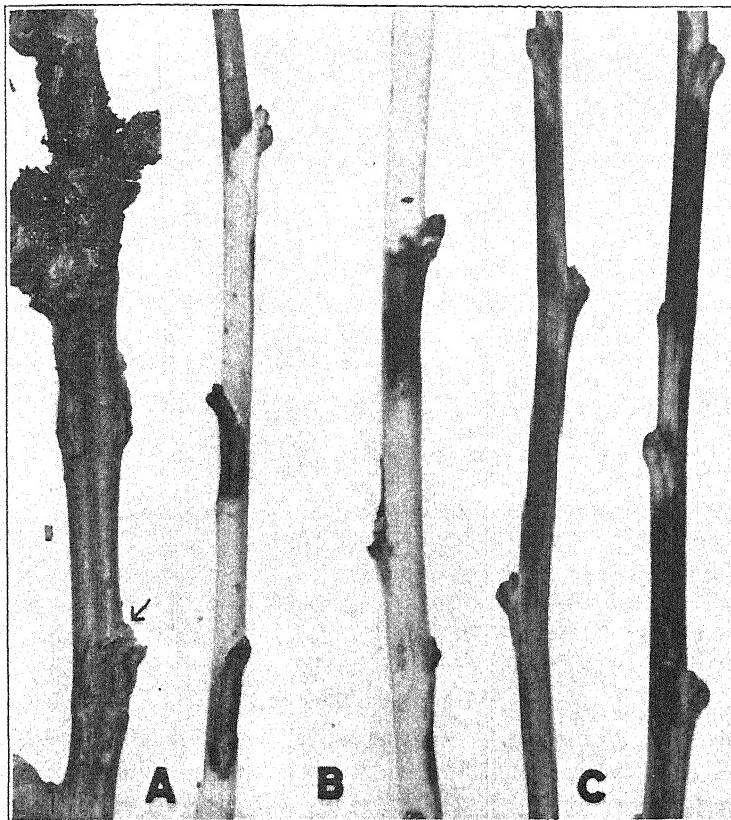
Fig. 3.—Bacterial gummosis. *A*, infection of a two-year-old Bing cherry branch through dormant buds. The remains of a bud and the accompanying canker are indicated by the arrow. *B*, the disease on current-year terminal growth of J. H. Hale peach. *C*, the disease on current-year terminal growth of apricot.

tion of green shoots was possible, the low humidity conditions of the Pacific Coast during the growing season would probably militate against an abundance of this type of injury." Within the last year and a half, however, two cases of blighting of green shoots by a bacterial organism have been found—one on apricot and one on peach (fig. 3B, C). On the apricot the disease was present as ill-defined, superficial, black streaks extending for some distance along the shoot. On the peach, the disease

had started at the axils of leaves and had extended downward as black, elliptical lesions bordered by a narrow band of water-soaked tissue.

Leaf-Spot.—Barss[5] mentioned a leaf-spotting of cherries produced by *Pseudomonas cerasi.* The spots were at first roughly circular and slightly water-soaked; but later the affected tissue browned and dropped away, producing a "shot-hole" effect. A spotting of cherry and apricot leaves was prevalent in California during the spring of 1932. These spots, which yielded bacteria, first appeared as minute brownish dots, each surrounded by a yellow halo. The tissues composing the dot and halo soon browned and fell away, producing a ragged hole (fig. 4).
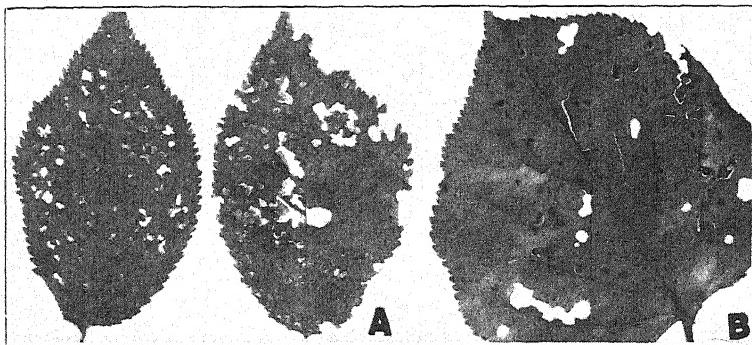
Fig. 4.—Symptoms produced by the gummosis bacterium: *A*, on cherry leaves; *B*, on apricot leaf. The abscission of the infected areas is clearly shown in *B*.

SUSCEPTIBILITY OF DIFFERENT KINDS AND DIFFERENT VARIETIES OF STONE FRUITS TO THE SOUR-SAP DISEASE

The stone fruits are not easily classified according to susceptibility under all situations in California. In the Sierra Nevada foothill districts, plums are severely attacked; in the valleys, the disease is usually not serious on either plum or prune varieties. By and large, the apricots, nectarines, cherries, and plums (including certain varieties used both as plums and as prunes) are more susceptible than the peaches or almonds. Little is known regarding the comparative susceptibility of different varieties of almonds; the disease has been found only on Nonpareil and Ne Plus Ultra.

The following discussion of susceptibility among varieties of cherries, plums, and peaches is based on observations in different parts of California and on records supplied by the Penryn Fruit Company, which owns or controls about a thousand acres of orchards in Placer County. The company's data consisted in a comparison of the percentage of trees lost during the serious outbreak of the disease in the spring of
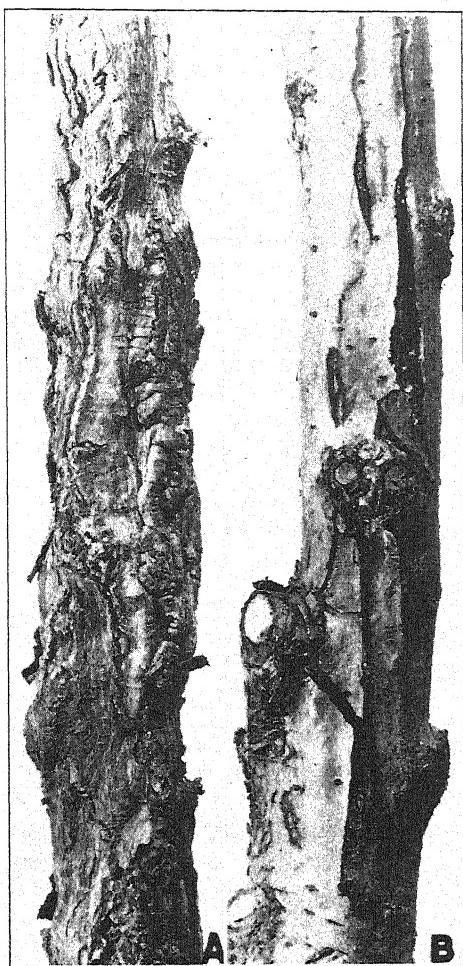
Fig. 5.—Reaction of two plum varieties to the disease. *A*, branch of Climax plum that has become gnarled through a vigorous healing along the later margins of cankers. These ridges of new tissue enable the distal branches to remain alive. *B*, badly diseased branch of President variety exhibiting comparatively little healing.

1930. The loss in the more important cherry varieties falls into the following decreasing order: Lambert, Napoleon (Royal Ann), Bing, Chapman, Republican, Black Tartarian. According to the Penryn Fruit Company, furthermore, the loss in the different varieties of plums falls into the following decreasing order (letters in parenthesis indicating European or Japanese stock): Duarte (J), President (E), Climax (J), Giant (J), Grand Duke (E), Santa Rosa (J), Tragedy (E), Burbank (J), Formosa (J), Diamond (E), Wickson (J), Gaviota (J), California Blue (E), Earliana (E), Sugar (E), Kelsey (J), and Beauty (J). Observations in the same locality both agree and disagree with these data. Whereas Duarte and President are unmistakably two of the most susceptible varieties and Kelsey seems most resistant, Burbank should probably be classed among the less susceptible and Beauty among the more susceptible. Of course, the data on losses reflect the ability of trees of certain varieties to withstand the inroads of the cankers even after infection. Though Beauty, for example, readily takes the disease, it does not show a high degree of mortality. In common with others (Climax, Santa Rosa, and Burbank), it exhibits marked ability to produce large amounts of new tissue along the canker margins, thus maintaining connections across the diseased area. The branch shown in figure 5*A* represents the healing in many trees of a young Climax plum orchard at Penryn. Despite severe infection, the mortality was low, and the trees were making good growth. Compare this with figure 5*B*, a badly diseased President plum branch exhibiting comparatively little healing ability, even though the canker had entirely girdled it. Such diverse responses may partially account for the different losses experienced in otherwise equally susceptible varieties.

Such peach varieties as Elberta, Phillips Cling, "Halford No. 2," and J. H. Hale appear from all available information to be severely affected; Alexander and Levy can be classed as moderately affected; Lovell, Tuskena (Tuscan), Yellow St. John (St. John), and Early Crawford are slightly affected.

Apricots are not planted on a commercial scale in Placer County. The few that are grown exhibit great susceptibility. Of the two most important commercial varieties, Blenheim was at first considered more subject to the disease than Tilton; but data collected in other districts have not substantiated this belief. In an orchard of two-year-old trees, where the disease appeared throughout the planting in the spring of 1932, 20 per cent of the Blenheim trees and 18 per cent of the Tiltons were affected. Whether loss of trees will be greater for one variety than for the other cannot be predicted at present.

SIMILAR BACTERIA ISOLATED FROM DIFFERENT TYPES OF INFECTION

Materials representing the gummosis type of limb canker were collected from widely separated parts of the state. This type of canker was found on apricot, cherry, peach, nectarine, almond, plum, and prune. In isolations made from the actively extending margins of such cankers, the bacteria appeared in most cases very similar when grown on potato-dextrose and nutrient agars. In isolations made from cankers representing the sour-sap type, the bacteria resembled those from gummosis cankers. Outside the Sierra Nevada foothill districts, the sour-sap type of cankers was found in only two places; on peach at Merced and on apricot at Madera, both in the San Joaquin Valley.

Isolations from several cases of blighted dormant buds, from the one case of blossom blight mentioned earlier, from green-shoot blight, and from leaf-spot, all yielded bacteria like those from gummosis and sour-sap cankers of limbs.

TWO TYPES OF BACTERIA DIFFERING IN PIGMENT PRODUCTION

Had the isolation work been discontinued after the first six months, the results would have strongly suggested that only one type of bacteria was constantly associated with both the sour-sap and gummosis types of cankers. In the spring of 1930, however, a collection of the gummosis type of canker on apricot, obtained from Orland, Glenn County, yielded a bacterium which, when grown on potato-dextrose and nutrient agars, resembled those obtained earlier in all but one respect—pigment production. The bacteria that had been obtained so consistently up to this time produced no pigment on potato-dextrose agar and only a slight lemon-yellow[5] discoloration of nutrient agar. The organism obtained from the Orland material, on the other hand, produced a brilliant green (lumiere green to apple green) discoloration of both media. In further collections from different orchards in the Orland district, the green organism was found exclusively. Since this time other samplings have been made in this locality with the same results. Shortly after the first isolation of the green organism, peach and cherry limbs bearing typical sour-sap cankers were collected in Placer County. Isolations yielded the green organism exclusively. That this organism was not constantly associated with the sour-sap cankers in Placer County was shown when many subsequent isolations yielded organisms of the first group only.

[5] All accurate color descriptions in this article are based on: Ridgeway, R. Color standards and color nomenclature. Published by the author, Washington, D. C. 1912.

Up to the present time isolations from all types of infections, including those of limb cankers, buds, blossoms, green shoots, and leaves, have revealed the green organism in but 10 to 15 per cent of the cases. Only once have the two types of organisms been found in the same canker. Needless to say, had materials been collected more frequently from the Orland district, the percentage for the green organism would probably have been much higher.

TABLE 1

SUMMARY OF THE NUMBER OF POSITIVE RESULTS OBTAINED FROM INOCULATING
YOUNG PLUM TREES ON JANUARY 15, 1931, AT PENRYN, CALIFORNIA

Source of bacteria	Variety inoculated	Group* of bacteria inoculated	Total number of inoculations	Number of successful inoculations	Per cent of inoculations successful
Sour-sap cankers	Wickson	White	63	63	100
	President	White	63	32	51
	Wickson	Green	24	20	83
	President	Green	24	18	75
	Wickson	Control	50	2	4
	President	Control	50	0	0
Gummosis cankers	Wickson	White	33	32	97
	President	White	33	27	82
	Wickson	Green	25	20	80
	President	Green	25	15	60

* White = bacteria that produced no pigment on potato-dextrose agar; green = bacteria that produced on potato-dextrose agar a green pigment, which diffused through the medium.

To facilitate references, these two groups of organisms will be designated in the table and the text as "white" and "green"—terms referring to pigment production on potato-dextrose agar only, since the more common "white" organism produced a lemon-yellow pigment in nutrient media. To designate the source of the culture, bacteria obtained from the sour-sap type of cankers will be assigned the letter "S"; those from the gummosis type, the letter "G."

BACTERIA FROM DIFFERENT SOURCES SIMILAR IN PATHOGENICITY

After some experimentation, a very successful method of inoculating branches and trunks of the host was devised. When a teasing needle had been passed tangentially through the bark, the bacteria in water suspension could be injected, by means of a hypodermic needle, into the hole thus made. Vaseline was then used to seal the holes, although this procedure was not necessary during the moist periods of winter and spring.

Pathogenicity of Bacteria from Gummosis and Sour-Sap Cankers.—
In studying the different isolations from the standpoint of pathogen-
icity, comparisons were made between bacteria of the two groups (white

TABLE 2

RESULTS OF INOCULATING THE TWO GROUPS OF BACTERIA FROM THE TWO TYPES OF
CANKERS INTO YOUNG PLUM TREES AT PENRYN, CALIFORNIA; 1931

Original source of bacteria	Group* of bacteria	Inoculation No.	Variety inoculated	Length† of cankers (inches)
Sour-sap cankers	White	1	Wickson	1–4
		2	Wickson	1–10
		3	Wickson	10–12
		4	Wickson	1–4
		5	Wickson	2–12
		6	Wickson	6–8
		7	Wickson	5
		8	Wickson	1–2
		9	Wickson	3–5
		10	President	3–4
		11	President	1–4
		12	Grand Duke	1–4
		13	Grand Duke	4–8
		14	Grand Duke	1–2
		15	Grand Duke	1–7
		16	Grand Duke	2–5
	Green	17	Wickson	1–2
		18	Wickson	1–5
		19	President	3–5
		20	Grand Duke	1–3
Gummosis cankers	White	21	Wickson	1–4
		22	Wickson	4
		23	Wickson	1–2
		24	Wickson	6
		25	Wickson	1–4
		26	President	2–4
		27	Grand Duke	1–2
		28	Grand Duke	2–7
		29	Grand Duke	4–5
	Green	30	Wickson	1
		31	Wickson	2–4
		32	President	3–5
		33	Grand Duke	1–4
	Control	34	Wickson	0
		35	Wickson	½
		36	President	0
		37	Grand Duke	0

* White=bacteria that produced no pigment on potato-dextrose agar; green=bacteria that produced
on potato-dextrose agar a green pigment, which diffused through the medium.
† Measurements of cankers were made 66-78 days after inoculations.

and green) and bacteria from the two types of cankers (sour-sap and
gummosis). During January, 1931, a total of 510 inoculations were
made into 120 trees of the President, Wickson, and Grand Duke vari-

eties of plums. Table 1 summarizes the number of positive results secured by March 22, the time of examining the inoculations. Table 2 gives the measurements of the cankers produced by the various cultures. The first table shows little difference in the percentage of successful inoculations between similar bacteria from the two types of cankers or between the two groups of bacteria. As to length of cankers (table 2), no significant difference appeared between bacteria of the same group
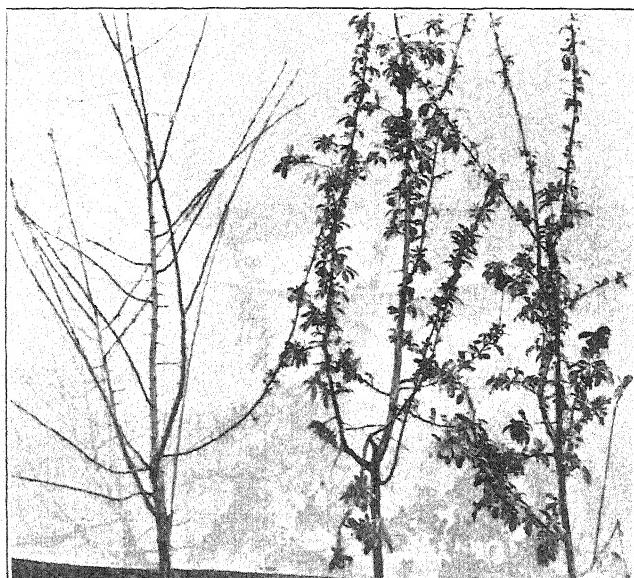
Fig. 6.—Sour-sap in young President plum trees. The tree on the left was inoculated with a white-S bacterium; the two trees on the right were not inoculated. No gum was exuded from the diseased tree.

even when obtained from the different types of cankers. There is a slight suggestion, however, that the green bacteria did not produce such extensive cankers as the white. Though a detailed description of the cankers will follow under a later heading, it may be said here that bacteria from gummosis and sour-sap cankers produced similar symptoms; nor was there evidence that bacteria of the two groups (white and green) produced different types of cankers. Figure 6 shows the results of inoculating a young plum tree with a white-S bacterium in February, 1932. While a large portion of the trunk was involved within a month, the tree survived the following summer, but died in the spring of 1933.

On January 14, 1932, twenty-five inoculations were made with each of the four cultures (white-S, white-G, green-S, and green-G) into six-year-old Bing cherry trees at Davis. By March 3, definite sunken areas

4 to 5 inches long and 2 to 3 inches wide had formed around the inoculation points. Gum was being exuded profusely from cankers produced by each culture. In several cases the cankers had, by extending in a spiral manner, completely encircled the branch. Here, again, bacteria of the two groups (white and green) and those from the two types of cankers (S and G) produced identical symptoms.

Next, inoculations were made into both peaches and apricots, and typical gummosis cankers were produced by each of the different cultures. On February 11, 1932, an exceedingly successful series of inoculations was made into trees of the Phillips Cling variety of peach and the Blenheim variety of apricot. Within two weeks definite sunken areas from 1 to 2 inches long had formed around the inoculation points, gum cavities had appeared along the margins of the cankers, and large globules of gum had been exuded through the inoculation wound. Within a month many cankers had become 4 inches long; a few had girdled branches 2 inches in diameter; and secondary infections, in every respect similar to natural infections, had appeared on branches below the inoculation points.

During the fall and winter of 1932–33, inoculations were made with single-cell cultures[6] of both the white and green organisms; these showed conclusively that unmixed cultures of each of the two types produced cankers on cherry and plum trees.

Inoculations of Dormant Buds.—Although most of the work has been concerned with the production of limb cankers, a few inoculations of dormant buds were made. Positive results have been obtained only with the white bacteria. This work, however, is probably not extensive enough to prove that the green bacteria are incapable of blighting dormant buds.

Inoculations of Green Shoots.—After the publication[27] of the comparison of *Pseudomonas prunicola* with a white-G bacterium designated as 357, an attempt was made at Berkeley to produce a blighting of green shoots with a number of the isolations, including *Ps. prunicola*. In a few cases, blighting of the growing tip for a distance of ½ to 1 inch was obtained; but there was no extensive blighting of the twig like that secured by Wormald[30] with *Ps. prunicola*. More severe blighting would probably result if other varieties of plums and trees in more succulent conditions were used, since Wormald[30] found extensive symptoms only on vigorously growing green shoots.

Pathogenicity of Bacteria Obtained from Dormant-Bud Blight and Green-Shoot Blight.—Inoculations and reisolations have positively dem-

onstrated that bacteria obtained from blighted dormant buds and green shoots can produce cankers as extensive as do bacteria from limb cankers. Two cultures, both of the white group, isolated from blighted dormant buds of apricot in the fall of 1929, have repeatedly produced cankers on limbs of plums and cherries.

GUMMOSIS AND SOUR-SAP TYPES OF CANKERS PRODUCED BY INOCULATION WITH THE SAME BACTERIUM

Descriptions of the cankers produced by the inoculations reported in tables 1 and 2 are presented here for comparison with results obtained at Davis. There was a marked contrast between cankers on the Wickson variety and those on the President. Whereas the majority of the former were advancing along the cambium, the latter were confined almost entirely to the outer phloem and cortex. Those on Wickson were gumming freely, while those on President were not producing gum. The cankers on Grand Duke trees were of an intermediate nature : some were advancing along the cambium, others through the phloem; some were gumming, while others were not. A comparison of these cankers with those from natural infection gave convincing evidence that the sour-sap type of symptom was being produced by the inoculations in President and Grand Duke. Figure 7C shows the diffuse nature of the canker produced on President by a bacterium of the white-S group; figure 2D, the same condition resulting from natural infection. The cankers on Wickson, on the other hand, in the exudation of gum and in the formation of well-defined necrotic areas, exhibited the gummosis type of symptoms (fig. 7A, B).

Symptoms of the diffuse, at first superficial, type were repeatedly obtained by inoculations of plums during the winter and spring of 1931–32. Inoculations made during January and February produced, on the whole, cankers that did not gum; those made during March and April produced cankers that gummed rather profusely in certain cases. The important point to remember here is that the same bacteria produced at different times symptoms varying as widely as those produced by natural infection. These variations were not surprising after a study was made of canker development throughout the winter and spring. During the winter (December and January) the cankers progressed, in the main, as water-soaked streaks. At this time there was very little uniform browning of the tissues between the streaks. Later (March and April), however, the tissues between the streaks browned more rapidly, producing a well-defined necrotic area—one closely similar to the gummosis types of cankers. Substantially the same thing has been found in

the study of cankers from natural infection. The difference in the amount of gum exuded by cankers produced in January and in April may result partially from the difference in the rapidity of necrosis and partially from the difference in the condition of the host.
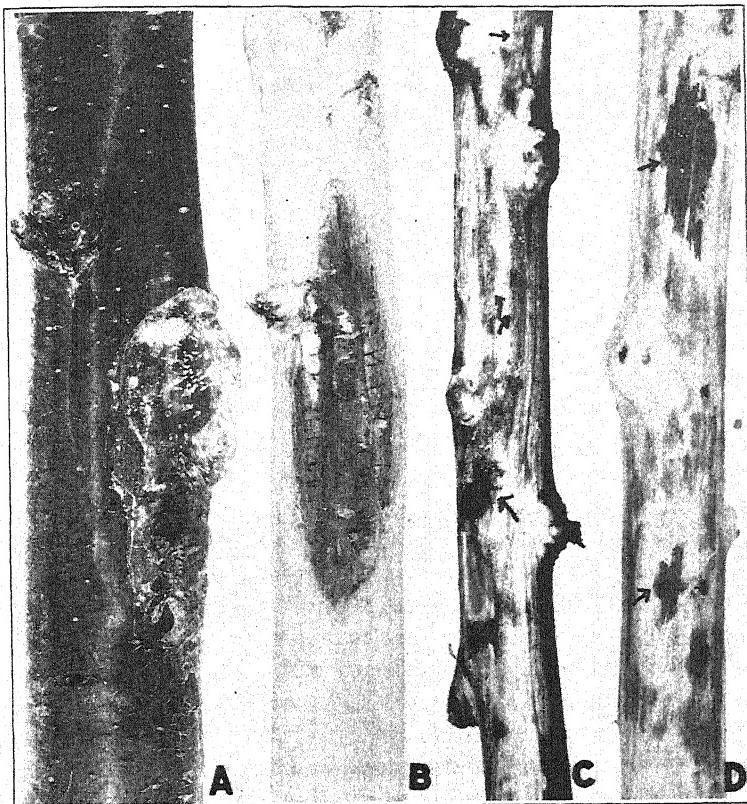
Fig. 7.—Various symptoms produced by the same organism (white-S). *A*, profuse exudation of gum from a canker on a Blenheim apricot branch. *B*, the same canker, somewhat reduced—a well-defined, elliptical, necrotic area representative of the gummosis type of symptom. *C*, a very diffuse diseased area representative of the sour-sap type of symptom. The small necrotic zone surrounding the inoculation point is indicated by the lower arrow. The watersoaked, slightly brown streaks extending above and below the inoculation point are indicated by small arrows. *D*, two midwinter inoculations resulting in slight necrosis even though the bacteria had invaded a large area. Bands of pigmented cortical tissue indicate the invaded zone.

In several instances, the same isolation produced different symptoms upon being inoculated into plums at Davis (on the floor of the Sacramento Valley) and into plums at Penryn (about 40 miles from Davis in the foothills of the Sierra Nevada mountains). One case in point was a series of inoculations made with a white-S culture into President

plums at Davis on October 23, 1931, followed by a series into the same variety at Penryn on October 24. Cankers that gummed freely, forming well defined, elliptical, necrotic areas, developed at Davis, while those produced at Penryn were small necrotic areas bordered by wide zones of loosely connected, only slightly brown, water-soaked streaks that did not exude gum.

GUM EXUDATION AND NECROSIS AT A MINIMUM IN CONTROL PUNCTURES

Although nothing has been said so far regarding control inoculations, large numbers were included in each experiment. When five or six-year-old trees were inoculated, 10 to 15 control punctures were made on one or two limbs of each tree. When smaller trees were used, the control punctures were made on trees located at intervals in the inoculated row. In the experiment listed in table 2, 150 control punctures were made on 30 trees. The rarity with which symptoms similar to those produced by inoculation developed in these control punctures is important. Only 2 of the 150 developed such symptoms, and this infrequent occurrence of natural infection in the controls held throughout the inoculation work. In most cases, necrosis of tissue surrounding the needle punctures was at a minimum: no brown streaks occurred either in the bark or along the surface of the sapwood, a condition likewise true of inoculations made with the pathogen at a season unfavorable for canker development.

The rare occurrence of abundant gum in these control punctures was interesting because gumming of apricot and cherry inoculations seemed closely correlated with the presence of a pathogen. It should be remembered that inoculation wounds were very small, a fact that probably contributed somewhat to the infrequency of gum production in the control punctures, inasmuch as mechanical wounding alone is said to cause gumming of stone-fruit trees (Butler[11] and others). Small beads of gum were sometimes exuded from the control punctures in apricot, peach, and cherry trees; but they were never so large nor so frequent as from trees inoculated with the pathogen. On these hosts the pathogen almost always caused profuse gumming. On plum, control punctures rarely produced gum.

CONSTANCY OF THE TWO TYPES OF BACTERIA IN REISOLATIONS

In 1931, reisolations were made from representative cankers produced by the series of inoculations presented in table 2. These were compared with the original cultures, and a number of each were used in inoculations the following winter. Where the green group of organisms

was inoculated, the same type of bacterium was obtained in the reisolations. Similarly, bacteria of the white group were obtained from cankers that had been produced from inoculation with this group. In this respect, the results were so consistent that during the winter of 1931–32 the following experiment was devised to study further the constancy of the two strains. Young President plum trees that had been growing for a year in pots at Berkeley, well isolated from any known source of the disease, were divided into six lots of ten trees each. Each lot was inoculated on October 7 with one of the following isolations: white-S-1, white-S-2, white-G-1, white-G-2, green-S-1, and green-G-1. The groups of trees were then placed out-of-doors 10 feet from each other, in order to minimize spread of the bacteria by splashing rain. Cankers from 2 to 4 inches long were produced by the majority of inoculations. After two months, five isolations were made from each canker. The bacteria were then carefully studied by plating in potato-dextrose agar. The results agreed closely with those obtained earlier: cankers produced by the green organisms yielded similar organisms in reisolations, while those produced by the white organisms yielded bacteria resembling those used for inoculation. Although this experiment points to a high degree of stability in the character of pigment production, much more work should be done along this line. Trees of different kinds, and different varieties of stone fruits growing in different localities, should be inoculated with the two groups of organisms; reisolations should be studied carefully.

MORPHOLOGY AND STAINING REACTIONS OF THE BACTERIA

In an earlier publication[27] the writer reported that culture 357 from gummosis cankers and *Pseudomonas prunicola*, as well, appeared to be Gram negative when a modification of the staining technique recommended in the S. A. B. Manual[23] was employed. When this procedure was followed in staining bacteria of the green group from both types of cankers and the white isolation from sour-sap cankers, these also were found to be Gram negative, as was further evidenced by their ability to grow well on a medium containing 1 part in 10,000 of gentian violet. The discussion of these tests will follow under an appropriate heading.

In the same publication the measurements of the bacterium 357 (white-G) and *Pseudomonas prunicola* (culture received from Wormald) were considered to exhibit no constant differences. In the present work organisms from these two isolations were again measured in comparison with isolations of the green group and with the white isolation

from sour-sap cankers. Table 3 summarizes the results of measuring 400 bacteria of each isolation. The fifth column of the table shows the probable error for each mean length. A statistical treatment of these results by calculating, for any two sets of measurements, the ratio of the mean difference to its probable error, reveals significant differences in length between *Ps. prunicola* on the one hand and white-G, white-S, and green-S on the other. That is to say, such a statistical treatment shows significant differences between these particular sets of measurements. One should not infer that these differences definitely separate the bacteria of the different isolations on the basis of length, particularly in the

TABLE 3

MEASUREMENTS OF BACTERIA

Group of bacteria*	Extremes of width	Extremes of length	Mean width	Mean length
	microns	*microns*	*microns*	*microns*
White-S	0.35-0.79	0.83-2.76	0.62	1.63±0.0121
White-G	0.35-0.79	0.83-3.03	0.55	1.67±0.0144
Green-S	0.35-0.79	0.83-2.76	0.57	1.62±0.0129
Green-G	0.35-0.79	0.83-3.03	0.62	1.72±0.0134
Pseudomonas prunicola	0.35-0.79	0.83-3.03	0.68	1.80±0.0143

* White=bacteria that produced no pigment on potato-dextrose agar; green=bacteria that produced on potato-dextrose agar a green pigment, which diffused through the medium. S=bacteria from sour-sap cankers; G=bacteria from gummosis cankers.

face of results presented in the earlier publication,[27] which showed equally great differences between measurements made of the same bacterium taken from different-aged cultures. For instance, the length of *Ps. prunicola* from a 48-hour-old culture averaged 2.1 μ; that from a 12-hour-old culture, 1.8 μ. With the results of this earlier work as a background, the data in table 3 can be interpreted as indicating that the bacteria are fairly similar in length, averaging from 1.6 to 1.8μ.

In the earlier publication[27] the writer states that both the bacterium designated as 357 and *Pseudomonas prunicola* commonly occurred in pairs. At times the former had been seen to produce long chains of cells similar to those reported by Wormald[30] for *Ps. prunicola*. This feature has since been exhibited by all the isolations in table 3 and by a single-cell culture of green-G. No attempt has been made to study the cause of the phenomenon.

Motility of the organisms in all the cultures in table 3 has been observed a number of times by means of dark-field illumination. Young, vigorously growing bacteria stained by Leifson's method were seen to bear from one to three polar flagella. The same staining technique demonstrated that many of the organisms in smears of each of the cultures were capsulated.

CULTURAL STUDIES

Methods.—Although certain methods employed in the cultural work will be described under the headings to which they pertain, a few may be mentioned here. Beef extract broth was prepared according to the formula in the S. A. B. Manual.[23] In addition to peptone and beef-extract, dextrose at the rate of 10 grams per liter was added. Beef-extract agar was made by adding 15 grams of agar per liter to this broth.

The synthetic media used in the carbon source studies were as follows:

Basal medium No. 1: ammonium dihydrogen phosphate, 0.5 gram; potassium chloride, 0.2 gram; and calcium chloride, 0.01 gram per liter.

Basal medium No. 2: potassium dihydrogen phosphate, 1.0 gram; magnesium sulfate, 0.5 gram; potassium chloride, 0.5 gram; sodium nitrate, 2.0 grams; and ferrous sulfate, 0.01 gram per liter.

These media were adjusted to pH 6.6 to 7.0 by addition of sodium hydroxide; then the desired carbon compound was added at the rate of 10 grams per liter. After tubing, the media were sterilized at 8 pounds pressure for 20 minutes.

Although basal medium No. 2 supported growth of all organisms better than did No. 1, it was less satisfactory in obtaining pH readings by the colorimetric method, inasmuch as the bacteria produced in the medium a pigment that hindered close matching with the standards.

Three different cultures of each of the two groups of organisms from the gummosis cankers and also from the sour-sap cankers were used in most of the culture work. *Pseudomonas prunicola*, sent to the writer by Dr. Wormald in the early part of 1931, was included in all the tests. Unless otherwise stated, all culture work was carried out at a temperature of 25° C.

Beef-Extract Agar.—It was on beef-extract and potato dextrose agars that the difference in pigment production between bacteria of the white and green groups was first seen. Although the growth characteristics of the bacteria on these media have been mentioned earlier, they will be summarized here.

Bacteria of the white group and *Pseudomonas prunicola*, when streaked on slants of this medium, produced a flat, slightly grey to colorless growth, the margin of which was lobed and the lobes in turn finely toothed. The surface of the growth was often marked by minute pits. The consistency was butyrous. After the bacteria had been growing for several days, the medium was discolored a lemon chrome to lemon yellow. No noticeable difference was observed between bacteria from the gummosis type of canker and those from the sour-sap type.

Bacteria of the green group produced growth similar in physical characteristics to that of the white group. The distinguishing feature, however, was the production by the green group of a lumiere green discoloration of the medium. On the whole, bacteria of the green group from the two types of cankers were indistinguishable, although one isolation from sour-sap cankers produced a lumiere green pigment that changed to a brown after several days, while the pigment of the other isolations remained green.

Beef-Extract Broth.—Bacteria of both groups and from both types of cankers produced in this medium a uniform cloudiness, followed by the slow accumulation of a granular sediment. Those of the green group tended to produce a fairly distinct surface film; those of the white, a slight film that easily fragmented when the tube was disturbed. The same difference in pigment production was observed in this medium as on the agar. Bacteria of the white group produced the lemon-yellow discoloration; those of the green, the distinctly green pigment. *Pseudomonas prunicola* was indistinguishable from bacteria of the white group.

Potato-Dextrose Agar.—Here, again, the physical characteristics of all bacteria were similar. The growth was flat, grey, glistening or (at times) glazed; and the margins were unevenly lobed. A zonated condition that often appeared near the margins apparently resulted from the uneven thickness of the growth. The consistency was butyrous. In pigment production, however, bacteria of the two groups separated themselves very sharply, those of the green group producing an intense yellowish green (lumiere green to apple green), those of the white no pigment. Bacteria from the two types of cankers were similar in all respects. As was mentioned before, certain of those from sour-sap produced the green pigment and were placed in the green group, while others produced no pigment and were placed in the white group.

Czapek's Agar.—All isolations of both groups of bacteria grew very vigorously on this agar. The bacterial mass possessed a more liquid consistency than on any of the agars mentioned above. It was slightly raised and glassy, with almost entire margins. Bacteria of both groups produced a distinct yellowish-green discoloration of the medium, although that produced by the green group was more intense.

Uschinsky's Medium.—Only fair growth was made by the bacteria in this medium. A uniform cloudiness of the medium was followed by a slow accumulation of a flocculent sediment, but no pellicle. Here, again, bacteria of both groups produced the distinct yellowish-green pigment, that of the green group being the more intense.

Malic Acid Agar.—This medium was prepared by adding 10 grams of malic acid and 15 grams of agar to 1,000 cc of basal medium 1. In this

case the physical characteristics of the bacterial growth were similar in all isolations from both types of canker. *Pseudomonas prunicola* differed only in making a less luxuriant growth than did the others. The growth along the streak was flat, glistening, glassy, with a slight buff tinge. The margins were entire or were only slightly lobed. In one experiment *Pseudomonas prunicola* and bacteria of the white group had not produced pigment by the end of 7 days, while those of the green group had produced the characteristic yellowish-green discoloration of the medium. In two later tests, however, bacteria of the white group produced within 8 days a greenish-yellow discoloration of the medium, though never so intense as that produced by bacteria of the green group.

Salicin Agar with Sodium Nitrate.—This was basal medium 2 with 10 grams of salicin per liter. All cultures made similar growth on slanted tubes of this medium. The growth was colorless, flat, filiform, and butyrous in consistency, with margins slightly lobed and crinkled. Only a slight decrease in pH of the medium was noted. All organisms produced a greenish pigment that diffused through the medium, those of the green group causing the most intense color.

Salicin Agar with Sodium Asparaginate.—Basal medium 2 was used, with sodium asparaginate substituted for sodium nitrate and with 10 grams of salicin as the carbon source. Somewhat poorer growth occurred on this than on the medium last discussed. The physical characters of the growth of all the cultures was the same; there was a difference, however, in pigment production, in that bacteria of the green group produced the characteristic green pigment, while those of the white group imparted a slightly brownish tinge to the medium.

Gentian Violet Bile Agar.—This medium was made by adding gentian violet (0.1 gram per liter) and Bacto-Oxgall (10 grams per liter) to beef-extract agar. When this medium was inoculated with a water suspension of the organisms and plates were poured, colonies appeared at 25° C within two or three days. The colonies of *Pseudomonas prunicola* and those of the white-G and white-S attained a size of 2 to 3 mm. The colonies were flat near the margin but were slightly raised in the centers. The gentian violet was present in the center of the colony as a well-defined, violet-colored disk surrounded at the periphery by a zone of unstained growth. The colonies of green-G and green-S were similar except that in the former the accumulation of stain was practically uniform throughout the colonies. The vigorous growth which the organisms made on this medium is considered good evidence that they are Gram negative.

Basic Fuchsin Sodium Sulfite.—This medium was made by adding 5 cc of a 5 per cent alcoholic solution of basic fuchsin and 35 cc of a 10 per

cent aqueous solution of sodium sulfite to basal medium 2 containing sucrose or dextrose and agar. Plates were poured, and the organisms in water suspensions were placed at six different points in each plate by means of a platinum loop.

On the plates containing dextrose as the carbon source all the organisms produced pink, circular, concentrically ridged growths. The agar surrounding the bacterial growth was partly decolorized. No distinction could be made between bacteria of the two groups. On plates with sucrose as the carbon source, all the cultures produced similar types of red colonies, but those of the white group from both sour-sap and gummosis cankers were a more intense red. In other words, the white bacteria produced a more pronounced Endo reaction.

Liquefaction of Gelatin.—These experiments were carried out at 19° C. All cultures under test liquefied the gelatin. The growth of the bacteria was, from the first, confined to the upper portion of the stab near the surface. Liquefaction proceeded in a stratiform manner at a rate that differed somewhat among the different isolations. The green group from gummosis cankers produced a more rapid liquefaction than the others, completely liquefying a column of gelatin 47 mm long in 30 days. This culture further differed from the others, including *Pseudomonas prunicola*, in producing a light yellow-green pigment in the medium; none of the other cultures produced pigment in these experiments.

Reaction in Milk.—Fresh skimmed milk was divided into two lots; to one was added 50 cc per liter of a saturated aqueous solution of litmus; to the second, 2 cc per liter of a saturated alcoholic solution of methylene blue. The material was then tubed and sterilized at 100° C for 15 minutes on each of 3 successive days.

Within a few days all the cultures produced in the litmus milk a definite alkaline condition, followed by a gradual peptonization. In these processes, cultures of the green organism from gummosis cankers were more active than any of the rest. By the end of 16 days, however, all cultures had completely peptonized the milk and had considerably decolorized the litmus, the green-G bacteria being more active than the others in the latter process. Throughout the experiment a white granular sediment gradually collected in all tubes except the checks. As no acid was formed in the milk, this sediment could not have been acid curd; nor did it have the characteristics of a rennet curd. It was thought to consist of materials thrown out of suspension by the change in pH.

The methylene blue was reduced by all the bacteria. In this respect, *Pseudomonas prunicola* was the slowest, and green-G the fastest. During the reduction the methylene blue changed from a beryl blue to a

Venice green. The green-G bacteria differed from the rest in produc-
ing a slight greenish cast in the cleared zone at the surface of the liquid.

Starch Hydrolysis.—Two types of starch agar were prepared as fol-
lows: (1) beef-extract broth (without dextrose) plus soluble starch at
the rate of 10 grams per liter, as directed in the Manual of the Society
of American Bacteriologists;[23] and (2) basal medium 2 plus 10 grams
per liter of soluble starch. The bacteria in water suspension were
streaked on poured plates of these agars. After 24, 48, and 120 hours of
incubation, three plates of each isolation were treated with a saturated
solution of iodine in 50 per cent alcohol.

By the end of 24 hours, all cultures of both groups of bacteria from
both types of cankers had made visible growth on the beef-extract me-
dium. Iodine tests, however, up to the end of 120 hours failed to show
hydrolysis of starch by any of the cultures. In the case of the synthetic
medium, no culture had made any visible growth by the end of 120
hours, and consequently no hydrolysis of starch was found. Apparently,
therefore, these bacteria cannot hydrolyze starch to a degree measur-
able by the method employed. The growth of the organisms observed in
the beef-extract-starch agar probably resulted from the utilization of a
carbon source other than the starch—for example, the peptone.

Utilization of Various Carbon Sources.—Earlier in the work the fer-
mentation of various carbohydrates was studied in a beef-extract broth.
In the presence of peptone, however, the bacteria so greatly increased
the alkalinity as to counteract the acid produced from certain of the
sugars. The use of this medium was thereafter discontinued in favor of
either basal medium 1 or 2. The latter was preferred because it sup-
ported a more vigorous growth. The results with the two types of basal
media agree in all respects. Those reported herein are from the use of
basal medium 2.

The data in table 4 show that the organisms from the two types of
cankers and from the two groups exhibited no differences in their abil-
ity to ferment the various carbon compounds. They all produced acid,
but no gas, from xylose, arabinose, mannose, dextrose, levulose, galac-
tose, sucrose, mannitol, and glycerin. Certain inconsistencies on the
levulose medium led to experiments in which the sugar was sterilized by
filtration. A comparison of this medium with one in which the levulose
was sterilized by heating at 8 pounds' pressure for 20 minutes demon-
strated that the inconsistencies were caused by the heating. All bacteria
consistently produced acid from the filtered sugar. In the experiment
reported in table 4 the bacteria had made no visible growth on rhamnose
by the end of 10 days; other experiments have shown that such growth

appears only after a somewhat longer period and results in acid production. When lactose, maltose, trehalose, or raffinose was used as a carbon source, the bacteria made rather sparse growth. The data indicate that the pH of the media containing these four sugars was shifted into the higher ranges. After 10 days, none of the isolations had grown on media

TABLE 4

UTILIZATION OF VARIOUS CARBON SOURCES BY ORGANISMS FROM THE TWO TYPES OF CANKERS

Carbon source	Change* in pH after 10 days at 25° C				
	White–S	White–G	Green–S	Green–G	*Pseudomonas prunicola*
Xylose	+	+	+	+	+
Arabinose	+	+	+	+	+
Rhamnose	0	0	0	0	0
Mannose	+	+	+	+	+
Dextrose	+	+	+	+	+
Levulose	+	+	+	+	+
Galactose	+	+	+	+	+
Sucrose	+	+	+	+	+
Lactose	−	−	−	−	−
Maltose	−	−	−	−	−
Trehalose	−	−	−	−	−
Raffinose	−	−	−	−	−
Mannitol	+	+	+	+	+
Glycerin	+	+	+	+	+
Peptone	−	−	−	−	−
Sodium asparaginate	−	−	−	−	−
Sodium acetate	0	0	0	0	0
Sodium tartrate	0	0	0	0	0
Sodium succinate	−	−	−	−	−
Sodium citrate	−	−	−	−	−
Sodium malate	−	−	−	−	−
Sodium lactate	−	−	−	−	−
Sodium benzoate	0	0	0	0	0

* Plus sign = production of acid; minus sign = production of alkali; zero = no growth.

containing sodium acetate, sodium tartrate, or sodium benzoate as a carbon source, while abundant growth accompanied the use of sodium asparaginate, sodium succinate, sodium malate, sodium citrate, or sodium lactate.

Utilization of Various Nitrogen Compounds.—In this work the following nitrogen compounds were substituted for sodium nitrate in basal medium 2: ammonium sulfate, sodium nitrate, sodium nitrite, and sodium asparaginate. The carbon source was dextrose.

All the cultures, including *Pseudomonas prunicola*, readily utilized all the nitrogen compounds except sodium nitrite. Likewise all isolations of both groups and from both types of cankers, as well as *Pseudo-*

monas prunicola, produced similar changes in the pH of the media. On the ammonium sulfate medium they progressively increased the hydrogen ion concentration during the first week of growth and maintained this acid condition throughout the 18 days of the experiment. On media in which the nitrogen source was either sodium nitrate or sodium asparaginate, the hydrogen ion concentration likewise increased for the first week of growth; but reversion followed, until by the end of the 18 days the sodium nitrate medium was only slightly more acid than the controls, while the sodium asparaginate medium was distinctly more alkaline.

Influence of Culture Media on Pigment Production by the Bacteria.— As has been emphasized throughout this paper, the only basis on which the bacteria were originally separated into the two groups, white and green, was the differences in pigment production on potato-dextrose and beef-extract media. Bacteria of the green group produced a definitely green pigment on both media, while those of the white group produced a pigment (lemon yellow) only on beef extract. The experiments reported under the inoculation studies show that bacteria of the green group retained the ability to produce the green pigment in a passage through the host, while the white bacteria showed no tendency towards assuming this ability. Isolations made over a period of three years have only once revealed bacteria of both groups in the same canker. All these indications therefore point to a stability of the chromogenic characters that differentiate these two groups.

In all culture tests the production of pigment by bacteria of the two groups was carefully compared. The bacteria of the green group produced pigment in the greatest number of cases. For instance, in an experiment where various carbon sources were added to medium 1, the green bacteria developed pigment in the xylose, arabinose, and mannose media, while the white bacteria did not. On the other hand, in the sodium succinate medium, bacteria of both groups produced pigment very similar in quality if not in intensity. Although the two types of bacteria are dissimilar on beef-extract and potato-dextrose media, they apparently tend to be less so on other media.

TEMPERATURE RELATIONS OF THE BACTERIA

To study the effect of temperature on growth of the bacteria, two methods were employed. By the first, the turbidity produced in basal medium 1 at the different temperatures was compared with a set of standards made from varying amounts of barium hydroxide and sulfuric acid. The second method consisted in placing the bacteria on the

surface of potato-dextrose agar by means of a platinum loop, incubating the plates at the desired temperatures, and measuring the diameter of the colonies at intervals.

By the first method the greatest turbidity was obtained in tubes incubated at 25° C, although only slightly less appeared in tubes kept at 20° and 30° C. All cultures were similar in this respect. By the second method the increase in diameter of colonies of the green organisms was greatest at 30° C, while increase in diameter of the white organisms and *Pseudomonas prunicola* was greatest at 25° C. Here, again, growth was only slightly less rapid at 20° and 30° C in certain experiments. Apparently, although these experiments indicate that bacteria of the green group possess a slightly higher optimum temperature than those of the white group, both groups grow vigorously between 20° and 30° C.

IDENTITY OF THE CAUSAL BACTERIA

Before discussing the previously described canker-producing pathogens of *Prunus* species, we shall briefly summarize the results of comparing the green and white organisms. First, as to the comparison of the bacteria from the two types of cankers (gummosis and sour-sap), neither the pathogenicity nor the cultural studies seem to indicate that the two types of cankers are produced by different organisms. On the contrary, all evidence supports the conclusion that they are produced by the same bacteria. Second, as to the comparison of the green and white types of bacteria, certain features besides pigment production apparently differentiate these two types. One possible difference is that the green organism required a slightly higher temperature for optimum growth, although the evidence is by no means conclusive. Certain slight differences appeared in the production of surface films in liquid media and in the rapidity of gelatin liquefaction and peptonization of milk. Attention, however, is called to the fact that both types of organisms did liquefy gelatin and did show similar reactions in milk (for example, reduction of methylene blue). A difference hard to evaluate was found by growing the two types of organisms on a modified Endo medium: the white bacteria gave more pronounced Endo test than did the green. Contrasted with these differences are the numerous similarities existing between the bacteria of the two types. Their carbohydrate metabolism was strikingly similar, as was their utilization of nitrogen compounds. Both types of bacteria possessed polar flagella and capsules. Although bacteria of the several isolations differed somewhat in length, the green organisms were not consistently differentiated from the white. According to a study of pigment production on various media, though the two

types of organisms differ in pigment production on potato-dextrose agar, on other media they produced pigment differing only in intensity.

Probably the most convincing evidence of similarity between the two types of bacteria was afforded by the pathogenicity studies. On plums, cherries, apricots, and peaches, both types produced cankers in every way similar. Inoculations made during the winter of 1932–33, with single-cell cultures of both types of bacteria, proved conclusively that the similarity of symptoms obtained in earlier studies did not result from mixed cultures.

In short, the evidence seems to warrant the conclusion that the two types do not differ widely enough to be placed in different species. Such differences as were observed may have resulted from strains within the same species.

We shall now consider the literature on bacteria pathogenic to species of *Prunus*. Of these, *Bacterium pruni*, described by E. F. Smith[22] in 1903, and *Pseudomonas cerasi wraggi*, described by Sackett[19] in 1925, differ widely from the organism found in this work. Both these species of organisms are yellow on culture media and possess other characters not encountered in the canker organisms. Having obtained a culture of *Pseudomonas cerasi wraggi* from Sackett in 1930, the writer found that it not only had distinctive characters in culture media, but produced no cankers in several trials on either apricot, cherry, or plum trees.

In 1905 Aderhold and Ruhland[1] named *Bacillus spongiosus* n. sp. as the organism producing a serious cankerous disease of cherry trees in Germany. In later publications[2, 3] they showed that this organism possessed polar flagella, a fact that would place it in the genus *Pseudomonas (Bacterium)*. Although their description of this bacterium is complete in certain respects, many important details are lacking. The organism does not appear to have been studied by other European workers. Braun[9] and Pape,[16] who mention it in connection with a cankerous condition of cherries in Germany, have apparently never studied it apart from the host.[7] It remains in the literature, therefore, as a putative species; its pathogenicity appears to have been established, but it has not been fully enough described to permit comparison with other organisms.

Griffin,[13] in his publication on bacterial gummosis, names *Pseudomonas cerasus* as a new species, resembling *Ps. spongiosa* closely in many respects, but differing from this organism in its failure to produce vacuolate or spongy colonies on certain media and in its production of a

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[7] Neither in publications nor in correspondence with the author has Braun or Pape mentioned studies of *Pseudomonas spongiosa*. Neither was able to furnish a culture of the organism.

green pigment; the latter characteristic is not attributed to *Ps. spongiosa*. Although Griffin did not mention any difference in the length of *Ps. spongiosa* and *Ps. cerasi*, according to Aderhold and Ruhland's[3] measurements the former ranges from 1.6 to 4.0μ in length, while Griffin's[15] measurements for *Ps. cerasi* ranged from 1.5 to 2.5μ (majority 1.8 × 0.6). Still another difference exists in the descriptions of the two organisms : *Ps. spongiosa* is reported to produce ammonia; *Ps. cerasi* is not. There seems to be a reasonable doubt that the two organisms are identical, despite their similarity in many respects.

Wormald[30] named the organism that he found to be the cause of the green-shoot wilt, *Pseudomonas prunicola*. He states that his bacterium did not appear to be identical with *Ps. spongiosa*, from descriptions of which it differed as to colony characteristics, action on milk, vigor of growth in Uschinsky's solution, and reaction to stains. He did not mention the difference between the length of *Ps. spongiosa*, as reported by Aderhold and Ruhland, and that of his organism. As stated in the last paragraph, the measurements of *Ps. spongiosa* ranged from 1.6 to 4.0μ. Wormald[30] reports *Ps. prunicola* as ranging from 0.9 to 2.5μ in length. He does not mention whether or not *Ps. prunicola* produces ammonia in culture media; but tests made by the writer[27] indicated that it does not. In this feature, therefore, it further differs from *Ps. spongiosa*.

Since *Pseudomonas cerasi* is reported to produce a distinct green pigment in common culture media, while *Ps. prunicola* produced only a lemon-yellow, Wormald[30] considered the two as distinct. The writer[27] questioned the advisability of accepting Wormald's organism as a new species because, in a comparison, *Ps. prunicola* appeared very similar to the common gummosis-canker-producing organism of California, both in cultural characters and in pathogenicity to limbs of plum and cherry. Furthermore, the writer hesitated to accept *Ps. prunicola* as a new species because a bacterium had been found resembling both *Ps. prunicola* and the common gummosis strain in all tests tried up to that time, with the exception of pigment production. This second bacterium produced in potato-dextrose and nutrient media a distinctly green pigment (fluorescence) similar to that reported for *Ps. cerasi*.[5, 13] A further reason for suspecting that the common white organism resembled the green was Goldsworthy's[14] earlier work in California with two organisms, which were said to be similar in most cultural features but different in pigment production and in serological tests. Goldsworthy asserted that both organisms produced gummosis in stone-fruit trees. In view of this situation the writer deemed further work necessary to establish the relationship of *Ps. prunicola* and the two types of gummosis organisms to one another and to *Ps. cerasi*. The present investigation

further indicates that *Ps. prunicola* and the common (white) canker organism of California are identical.

Asserting that *Pseudomonas prunicola* alone was responsible for the green-shoot wilt, Wormald[30, 31, 32, 33] names a second organism, *Ps. mors-prunorum,* causing a canker disease prevalent in England. Although both organisms were capable of producing limb cankers, fruit-spot, and leaf-spot,[30, 33] and were similar in certain cultural features, he considered them separate species, inasmuch as *Ps. mors-prunorum* failed to produce pigment in nutrient broth and Uschinsky's solution, it increased the hydrogen-ion concentration in lactose media, and it remained alive only four to six days in a 5 per cent sucrose medium. Wormald's results, however, show that separate cultures of *Ps. mors-prunorum* differed among themselves almost as widely as did the two species. A careful comparison of the English canker organism and the bacteria described herein is obviously desirable.

Admitting the limitations imposed on a discussion of the relationships of two organisms that have not first been tested comparatively[8] in the same laboratory, the writer wishes to submit the results here presented as evidence that the green strain described is identical with *Pseudomonas cerasi.* The white organism, accordingly, would be considered a strain or variety of *Ps. cerasi* and might be designated as the variety *prunicola.*

Pseudomonas cerasi var. prunicola n. var.—A rod-shaped, aerobic, capsulated, Gram negative, motile (one to three polar flagella) organism, commonly occurring in pairs, occasionally forming long chains. Individual cells are 0.83 to 3.03μ long and 0.35 to 0.79μ wide, averaging 1.7 by 0.6μ. On nutrient agar it produces flat, butyrous, colorless to white colonies, the margins of which are finely lobed. A lemon-chrome to lemon-yellow discoloration of the medium occurs after the bacteria have been growing for several days. On potato-dextrose agar the colonies are whiter than on nutrient-agar; otherwise, the growth characteristics are about the same. No pigment is formed on this medium. The organism produces acid but no gas from arabinose, xylose, mannose, dextrose, levulose, galactose, sucrose, mannitol, and glycerin; it grows poorly on rhamnose. It decreases the hydrogen-ion concentration when growing on lactose, maltose, trehalose, raffinose, or peptone. Other carbon compounds utilized are sodium asparaginate, sodium succinate, sodium citrate, sodium malate, and sodium lactate; but apparantly not sodium acetate, sodium tartrate, or sodium benzoate. Starch is not hydrolyzed. No ammonia is produced, and nitrates are not reduced to nitrites. Ammonium sulfate, sodium asparaginate, and sodium nitrate are satisfactory nitrogen sources. The bacterium produces a moderately rapid stratiform liquefaction of gelatin stabs. In milk, there is first an increase in alkalinity, then a peptonization, but without the formation of curd. In this medium litmus is partially decolorized, while methylene blue is reduced. The optimum temperature for growth

[8] The author was unable to obtain either the original or a more recent isolation of *Pseudomonas cerasi* from Oregon.

of the bacterium is about 25° C. The organism is pathogenic to species of *Prunus* causing dormant-bud blight, green-shoot blight, leaf-spot, and limb cankers.

The varietal distinction is based largely on differences in chromogenesis. *Pseudomonas cerasi* Griffin produces on potato-dextrose agar a lumiere-green to apple-green pigment with fluorescent properties; *Ps. cerasi* var. *prunicola* forms no pigment on this medium. Differences in intensity of pigment are observable on other media, *Ps. cerasi* producing a more distinctly green color than *Ps. cerasi* var. *prunicola*.

According to some indications in the literature, it may become desirable to reclassify a number of closely related organisms that have heretofore been considered distinct species. Attention is called to Smith and Fawcett's[20] comparison of *Bacterium syringae, B. citriputeale*, and an isolation from stone fruits which they called *B. cerasi*.[9] These three organisms showed marked similarities in cultural characters and in their reaction on various hosts. Although Smith and Fawcett did not propose to group these organisms under one species, they stated that if this is ever done, *B. syringae* should be the species name.[10] Smith,[21] who has more recently compared Wormald's *Ps. prunicola* with *B. citriputeale*, finds the two very similar and probably identical. On the basis of his and Fawcett's earlier work he avers that the same relations exist between *Ps. prunicola* and *B. cerasi*.

Should the reclassification be undertaken, the gummosis-canker bacterium of stone-fruit trees would probably be included; it would then not retain the species name *B. cerasi*, since the species *B. syringae* antedates it by nine years.

NEW CANKERS APPEAR DURING WINTER AND EARLY SPRING

As the gummosis and sour-sap types of symptoms are evidently caused by the same bacterium, the two can be discussed as one disease. Although most of the following observations were made on plums in Placer County, supplementary inspections of peach, apricot, and cherry trees were carried out in other localities.

New cankers, appearing first as small, brownish flecks in the cortex of the bark, are extremely hard to detect. In winter, they are slow to develop such external symptoms as gummosis or cracking and sinking of the periderm; in spring, they exhibit these signs almost immediately.

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[9] Smith and Fawcett's culture of *B. cerasi* was obtained in California. After corresponding with Smith, the writer is convinced that it belongs to the white group described herein.

[10] Since the publication of Smith and Fawcett's work, Miss Charlotte Elliott, in her "Manual of Bacterial Plant Pathogens," Williams and Wilkins Co., page 217, has made *Bacterium citriputeale*, but not *B. cerasi*, synonymous with *B. syringae*.

Consequently, the time of their first appearance can be only approximately established.

During the season of 1929–30 an outbreak of disease was discovered in late January. A month later, the cankers were exuding gum or a watery material; by March many of them had girdled limbs and directly caused serious losses of peach, nectarine, and plum trees. During the season of 1930–31, two major outbreaks occurred. The first was discovered on January 9, just as numerous cankers began to appear a few inches below old diseased areas. Apparently they arose from infection by bacteria washed down in rain water from the old cankers. The second outbreak was found on April 1, 1931, when the diseased areas were producing large amounts of gum. Worse infection occurred during the season of 1931–32 than in either of the preceding seasons. The first cankers were noted on December 31; and from this time to February 1 numerous cases developed. Beginning again about the first week in March and continuing for approximately two weeks, new cankers appeared on apricot, peach, and plum trees, both in Placer County and in the Sacramento-San Joaquin valley area. Although, as a rule, not large enough to cause much damage during the spring, they constituted a danger the following season. No new disease was observed during the season of 1932–33 until February 15, when a moderate amount developed in pruning wounds.

As there is only fragmentary evidence regarding the length of incubation periods under various climatic conditions, the time when the different outbreaks were initiated cannot be determined. Since the disease appeared in midwinter and spring rather than autumn, a good deal of rain was apparently necessary for infection; or at least, infection occurred with greatest frequency during long rains.

EXTENSION OF ESTABLISHED, OR HOLD-OVER, CANKERS CONFINED TO WINTER AND EARLY SPRING

The diseased areas remaining in the tree from one season to another, in addition to serving as sources of abundant and readily available inoculum, are centers from which new tissue is invaded. Such cankers pass the summer in a quiescent state. Resuming activity in October or November, they continue to extend throughout the winter by means of small, slightly brown streaks. Sometime in early spring—generally late February—the tissue between the streaks begins to die; by March it turns uniformly brown and sour. If the streaks are numerous, necrosis continues up to the limits of the zone they occupy, forming well-defined cankers (fig. 7B); if they are few, only the center of the diseased area is

killed (fig. 2*D*), and the result is diffuse cankers. In April, canker extension begins to wane; and by May or June it stops or becomes too slow to be detected.

TABLE 5

PRODUCTION OF CANKERS BY PSEUDOMONAS CERASI VAR. PRUNICOLA AT DIFFERENT
TIMES OF THE YEAR

Date of inoculation	1931–32: On plum at Penryn			1932–33: On cherry at Davis		
	Inoculations producing symptoms	Average length 20 days after inoculation	Average temperature between inoculations	Inoculations producing symptoms	Average length 20 days after inoculation	Average temperature between inoculations
	per cent	*millimeters*	*degrees Fahr.*	*per cent*	*millimeters*	*degrees Fahr.*
April 7	0	0	64	....	....	....
June 5	0	0	72	....	....	....
August 18	22	3	92	....	....	....
September 4	29	2	69	....	....	....
October 1	25	4	68	....	....	....
October 11	....	....	....	40	3	61
October 20	82	76	59	....	....	....
October 31	75	32	70	83	14	56
November 15	....	....	....	87	8	58
November 18	80	7	46	....	....	....
December 1	....	....	....	83	4	40
December 2	81	16	43	....	....	....
December 17	54	6	44	....	....	....
December 29	....	....	....	89	4	41
December 31	69	12	47	....	....	....
January 13	79	6	41	....	....	....
February 2	84	9	45	93	10	44
February 17	....	....	....	100	19	47
March 8	92	50	57	....	....	....
March 22	87	30	60	100	5	55
April 12	63	14	56	....	....	....
May 10	0	0	68	....	....	....

THE SEASONAL ASPECT OF CANKER ACTIVITY IN RELATION TO TEMPERATURE AND RAINFALL

Barss[6] in Oregon and Goldsworthy and Smith[15] in California found that inoculations produced the disease only during winter and spring, thus establishing its seasonal character. The present investigation revealed a similar situation in the autumnal transition of cankers from a quiescent to an active state and in the springtime reversal of the process. In a search for causes of this phenomenon, such seasonal variables as temperature and rainfall were considered, particularly as the latter affects soil moisture. Certain facts appear to eliminate soil moisture as a possible cause. First, in two of the four years, canker activity began before the rainy season, at a time when the soil moisture was low. Second, although late summer irrigation increased soil moisture in certain orchards, no effect on canker activity was noticed.

For the purpose of collating canker development and temperatures, inoculations were made at intervals throughout the autumns, winters, and springs of two seasons. Table 5 contains data on average canker length, percentage of inoculations developing symptoms, and mean temperature between inoculations. The results of the 1931–32 experiment were as follows: no symptoms were produced in April and June, 1931; only a few small necrotic areas developed during August, September, and early October; but definite cankers resulted from late October and November inoculations. Then followed a period during December (1931), January, and February (1932), when a high percentage of the inoculations developed small but definite lesions. March and April, 1932, were favorable for rapid canker extension. No symptoms whatever attended the inoculations of May 10. The 1932–33 experiment gave essentially the same results, although, on the whole, the symptoms were less extensive than those of the year before. Larger cankers developed in inoculations of October 31 and November 15 than in those made either immediately before (October 1) or immediately after (December 1) these dates. Although a second December series produced only small lesions, a high percentage of the trials were successful. February and March, on the other hand, favored more rapid progress of the disease.

These experiments justify the conception, gained earlier by observation, that activity which begins each autumn in certain established cankers continues until spring. They go beyond the observations, in that the extension rate is shown to vary, decreasing in winter and increasing in spring. An examination of table 5 shows that these variations coincide with fluctuations of temperature. In 1931, for example, large cankers developed during late October and November at mean temperatures from 57° to 70° F; during December (1931) and January (1932) small ones developed at temperatures from 41° to 47° F. In 1932, likewise, larger diseased areas were produced at 56° to 58° F (October 31 and November 15) than at 40° to 41° F (December 1 and December 29).

The resumption of canker activity in autumn, however, cannot be explained as a direct response to temperature. To regard it as such, one must assume that a drop of 9° F in 1931 (from 68° in early October to 59° F in late October) and of 5° F in 1932 (from 61° in October to 56° F in November) caused the differences in size of cankers shown in table 5. On the contrary, two experiments, in which inoculated trees were held at 36°, 50°, and 65°–70° F, indicated that the greatest canker extension occurred at this last temperature and that 50° was definitely more favorable than 36° F.

The failure to obtain cankers during the higher temperatures of early

autumn may possibly be explained by the host's reactions to the diseased
areas. The few small lesions that formed in September and early Octo-
ber were promptly and effectively buried between new tissue; thereafter,
they made no noticeable progress. Even the smallest necrotic streaks
were surrounded by a meristematic layer of cells (fig. 8). In late Octo-
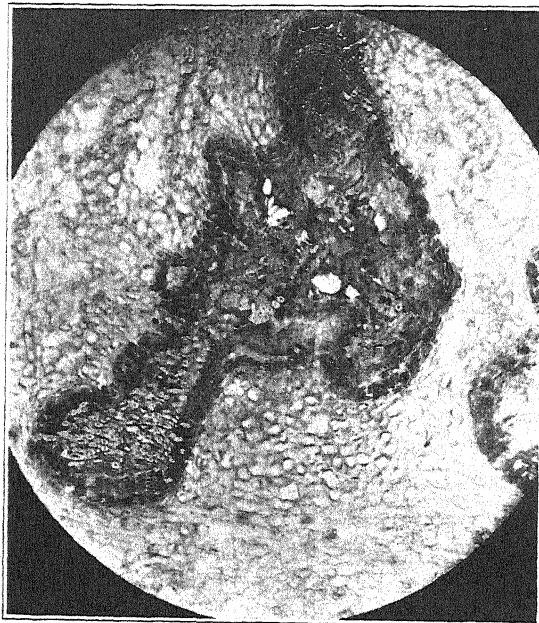
Fig. 8.—Cross section through a necrotic streak sur-
rounded by layers of meristematic tissue—a characteristic
of quiescent cankers.

ber or early November, the trees apparently lost this ability to respond
to the presence of cankers; they did not regain it until spring. In both
1931–32 and 1932–33, large cankers developed if inoculations were
made in late October or November, a time when the trees were incapable
of responding to wound stimuli.

If temperature influences the start of canker activity in autumn, it
must do so through its effect on the host. The literature dealing with the
relation of temperature to the rest period in orchard trees is scattered;
to review it would require more time and space than this brief discussion
affords.

SUMMARY AND CONCLUSIONS

Investigations, beginning with that of Brzezinski in Poland in 1902, followed by that of Aderhold and Ruhland in Germany in 1905, have shown that bacteria produce on *Prunus* spp. certain canker diseases, characterized by copious gum exudation. Whether the disease described in Poland is identical with that found in Germany cannot be determined from the literature.

Griffin in Oregon (1911) was the first American investigator to establish the bacterial nature of a cherry gummosis. Thus far, this disease has been reported only from states on the Pacific Coast, having been found in California by Barrett in 1916. In 1930, Goldsworthy and Smith described a cankerous disease attacking stone-fruit trees in the Sierra Nevada foothills. This malady, differing somewhat in symptoms from gummosis, was shown to result from bacterial infection, although the pathogen was not described.

In the present investigations, bacterial gummosis was compared with the disease described as "sour-sap" by Goldsworthy and Smith. The sour-sap disease is characterized by the failure of entire trees or portions of trees to produce leaves in the spring. Others start to grow, but the foliage suddenly wilts after the first warm days. The bark of limbs and trunks is girdled by ill-defined, brown, moist, sour-smelling necrotic areas.

The disease generally remains in the aboveground portion of the tree; no proved case has been found in the roots. A second striking feature is the lack of gum formation except on infrequent occasions.

The gummosis disease has been regarded as differing from the sour-sap in the presence of abundant gum and in better defined, deeper cankers. These characteristics differentiate the two troubles at certain periods but not at others. Such factors as time and kind of stone fruit appear to influence greatly the expression of the disease. Bacterial infection of leaves, green shoots, blossoms, and dormant buds is described; and its connection with the limb canker phase is discussed.

Apricots, cherries, and plums are apparently more susceptible to the sour-sap type of trouble than are peaches and almonds. Differences in varietal susceptibility are found, the Lambert cherry, the President plum, and the Phillips Cling peach being among the most severely affected; the Black Tartarian cherry, the Kelsey plum, and the Early Crawford peach, less affected. Certain plum varieties heal vigorously along canker margins, thus maintaining, across diseased areas, ridges of healthy tissue that enable the distal portion of branches to remain alive. Other varieties exhibit little healing ability.

Isolations from sour-sap and gummosis types of cankers yielded similar organisms. Two types of bacteria differing in chromogenesis were found: one group produced a distinct lumiere-green pigment on potato-dextrose agar; the other, no pigment. The latter type was found in from 85 to 90 per cent of the isolations. The chromogenic character of the bacteria apparently remained fairly constant, no noticeable change taking place in a passage through the host.

Inoculations, using single-cell isolations in certain cases, proved that both groups of bacteria were able to produce identical symptoms on plum, peach, apricot, and cherry limbs. Both groups of bacteria produced in certain instances the sour-sap type of cankers, in other cases the gummosis type. In 1931, parallel inoculations of President and Wickson plum trees produced, in the former, indefinite cankers that did not form gum, and, in the latter, well-defined cankers exuding abundant gum. This experiment and others in conformity with earlier observational results, proved that the sour-sap and gummosis symptoms are manifestations of the same disease. Factors other than cause appear to determine the type of symptom.

Cultural studies failed to reveal marked distinctions between bacteria of the two types. They were similar in size, in number and arrangement of flagella, in growth characteristics on various media, and in utilization of carbon and nitrogen compounds. They showed only slight differences in liquefaction of gelatin and in reactions on milk. The most consistent difference was intensity of pigment production, one group causing a lumiere-green discoloration of most media, the other forming a lemon-yellow pigment in certain media but not in others. On a medium containing basic fuchsin decolorized with sodium sulfite, both types of organisms accumulated the fuchsin in the colonies, but with different degrees of intensity. Some evidence, by no means conclusive, indicated that the green organism might require a slightly higher temperature for optimum growth than the white. In view of the pathogenicity studies, however, the two groups of organisms are not considered separate species, but probably represent strains of the same species.

The cultural studies showed, furthermore, that similar bacteria were obtained from the sour-sap and gummosis types of cankers. *Pseudomonas prunicola* Wormald was found to be identical with the white bacteria obtained from both types of cankers.

A review of the literature on other pathogens shows *Bacterium pruni* E. F. S. and *Pseudomonas cerasi wraggi* Sackett to differ widely from the organism herein described, and raises legitimate doubts that the bacterium mentioned in this paper is identical with *Ps. spongiosa* Aderhold and Ruhland, despite its similarity. The green strain, on the other

hand, appears identical with *Ps. cerasi* Griffin. Earlier studies had shown the marked similarity between the white strain and *Ps. prunicola* Wormald; the present investigation confirms this view. Evidently, therefore, the green strain is *Ps. cerasi*. The white strain should be called *Ps. cerasi* var. *prunicola* n. var., a description of which is included.

No opinion is expressed as to the relation between *Pseudomonas mors-prunorum* Wormald and the organism described herein. Wormald asserts that *Ps. mors-prunorum* differs from *Ps. prunicola*, which, as already shown, is probably identical with *Ps. cerasi* var. *prunicola*, the white strain mentioned in this paper.

If certain allied organisms such as *Bacterium syringae* van Hall, *Bacterium citriputeale* C. O. Smith, and *Pseudomonas cerasi* are reclassified, the status of the bacterium last mentioned will be affected.

New disease appeared during the winter and spring. As young cankers are extremely hard to detect, very little is known concerning the length of the incubation period under various climatic conditions.

After beginning activity in the autumn, established (hold-over) cankers continue to extend during winter and early spring; becoming quiescent in late spring, they remain so throughout the summer. Inoculations in early autumn and late spring produced only small lesions; those in late autumn and early spring, extensive cankers—further evidence that the disease has a marked seasonal character. Although temperature affects the rate of canker extension, it is apparently not directly responsible for failures to obtain marked symptoms in early autumn and late spring. On the other hand, according to certain evidence, the host itself may influence the disease, inasmuch as the lesions developed during these periods were effectively buried between new host tissue, while those initiated in late autumn or early spring were not.

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SULFURIC ACID AS A PENETRATING AGENT IN ARSENICAL SPRAYS FOR WEED CONTROL[1]

A. S. CRAFTS[2]

Sulfuric acid has been successfully used in the control of annual weeds and plant diseases in grain fields.[11] Åslander, in 1927,[1] reviewed briefly the earlier work and reported valuable experimental studies on the action of this chemical upon plants. He listed 53 weeds that have been killed and mentioned a few that did not respond to the acid. Most of the latter were perennials, grasses, and plants difficult to wet. He discussed the influence of soil moisture, relative humidity, and temperature upon the action of the acid and made histological studies on treated mustard leaves. Under the microscope he examined *Elodea* leaves in acid solutions. In all his work he compared sulfuric acid with iron sulfate. He found the acid much more rapid in its action upon the plant and explained its effectiveness in dry regions upon this basis. When the relative humidity was low, he found iron sulfate to crystallize on the leaves before penetration had taken place.

Sulfuric acid has proved useful in Arizona[3] against a number of weeds. Being produced as a convenient outlet for certain by-products of the smelting industry, it is relatively inexpensive. The chief drawback to its general use is its strongly corrosive action on metal equipment—a difficulty that must be overcome before it can serve the farmer in combating annual weeds.

More recently[5] sulfuric acid has been found useful as a penetrating agent in an acid arsenical spray that promises to become useful in controlling certain deep-rooted perennial weeds. The mechanism responsible for the action of this type of spray was described in 1927,[8] and further experiments were reported in 1930.[4] A later publication[5]

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cites plot tests that indicate the critical concentrations of acid and arsenic. All these reports emphasize the important role of acid in the penetration of the spray solution. Since the success of this method so largely depends upon the operator's understanding of its mechanics, the underlying principles might well be studied more thoroughly. The present paper describes the reaction of the plant to sulfuric acid, provides data for evaluating the various factors involved, and discusses the relation of these factors to actual spray practice.

THE REACTION OF PLANT CELLS TO STRONG ACID

Åslander studied the effect of sulfuric acid on *Elodea* leaves. He found that it rapidly stopped protoplasmic streaming but in no case caused plasmolysis. Being convenient, fairly uniform, and easy to observe, the same material was used in the present studies. *Elodea* leaves, mounted in water between a flat slide and a cover glass, were examined at a magnification of 900 (Zeiss apochromat N.A. 1.3, 90x and 10x compensating oculars). At this magnification the rapid flow of protoplasm could be distinctly seen, the small chondriosomes were very clearly differentiated, and the relation of the protoplasm to the slowly moving plastids could be studied. The nuclei were clearly defined.

Figure 1*A* diagrams a normal leaf cell of *Elodea*, with arrows denoting the location and direction of streaming. The narrow strands of protoplasm stretching across the vacuole exhibit rapid motion; but close observation shows that the chondriosomes, in addition to being carried along in this smoothly flowing stream, also have impressed upon them the slight jerky motion of thermal agitation. The vacuoles in healthy cells appear clear and free from particles.

After selecting a favorable location, usually at the base of the leaf, where the plastids are not numerous and streaming is active, a drop of sulfuric acid was applied to the cover glass, and the cells were watched closely. With 0.1 N acid the killing process occurs slowly, so that the different stages can be studied. With stronger acid the process goes on more rapidly, but is not essentially different.

The first sign of acid injury is a gradual slowing down of the flow along the outer surface of the protoplasmic strands. The cause, apparently, is not increased viscosity, for the thermal agitation of the chondriosomes continues unaltered even after unidirectional movement has ceased. As this slowing down proceeds toward the center of the strand, the chondriosomes farther within lose their unidirectional motion until flow stops and they exhibit only the jerky Brownian movement. This movement continues for some time after streaming has stopped and the

plastids have come to rest. The strands of protoplasm appear to thicken somewhat, while the parietal layer lining the wall assumes a rough, swelled appearance. The protoplasm apparently increases in viscosity with time as the Brownian movement finally slows and stops, and all structures previously in motion come to complete rest. Meanwhile very minute particles appear in the vacuole. As they first become visible
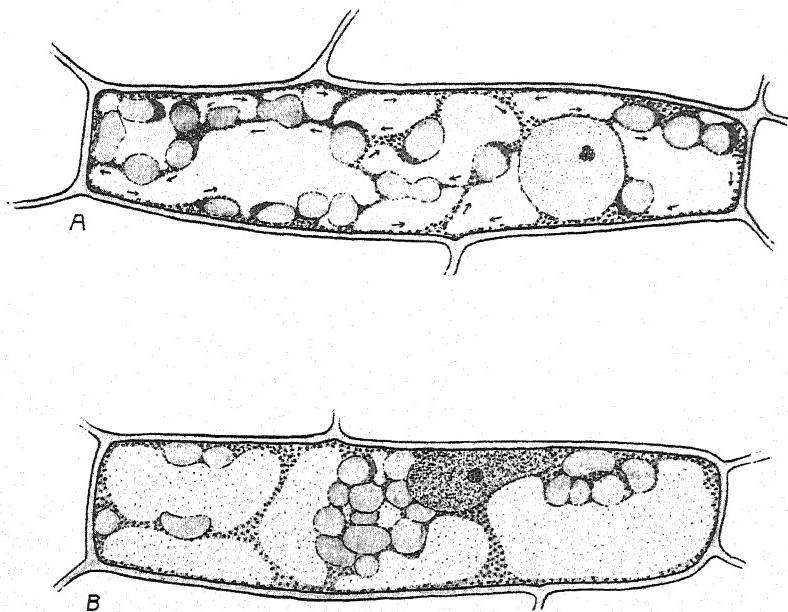
Fig. 1. The effect of sulfuric acid on *Elodea* cells; a comparison of *(A)* healthy, and *(B)* acid-killed cells.

they show very violent thermal motion; gaining in volume they become somewhat more sluggish. Their ultimate size approaches that of the chondriosomes, but they remain free moving within the vacuole. As the acid penetrates to the plastids, the green coloring matter turns to the light yellowish green, described by Åslander. These studies also substantiate Åslander's statement that no plasmolysis occurs in cells treated with sulfuric acid. The protoplasm is apparently killed and rendered permeable so rapidly that no withdrawal of water can take place. After killing with 4.0 N acid the protoplast slowly shrinks, resembling plasmolysis, but only after the cessation of streaming and the color change of the chlorophyll have shown that the cell is dead. Figure 1B shows a killed cell. The protoplasm, and especially the nucleus, appears granular. The plastids show the characteristic grouping described by Åslander. Chondriosomes are fixed within the protoplasm, while the vacuole is occupied by many minute, rapidly agitated particles.

STUDIES ON THE RATE OF KILLING OF PLANT CELLS BY STRONG ACID

Stiles and Jorgensen[13] found that plant tissues absorb hydrogen ions rapidly and that a simple exponential relation exists between absorption time and acid concentration.[14] The temperature coefficient for absorption within the range 0°–30° C was 2.2 for each 10°. On the basis of these two findings they suggest that this absorption is controlled by some chemical action in the cell.

Brenner,[2] conducting extensive studies on the permeability of plant cells to acids and bases, found that the toxicity of acids varied with the hydrogen-ion concentration.

Heilbrunn[6] reviewed the work of several investigators on the action of acids and alkalies upon protoplasm. He found general agreement on the observation that acids cause coagulation, an increase in the granules of the cell, and—in high concentrations—death.

These investigators were interested primarily in the reaction of plant tissues to relatively low concentrations of acid, and their experiments covered considerable periods of time. The present studies more directly concern the rapid killing of cells by concentrated strong acids.

The living protoplasm of the plant cell constitutes an extremely complex physico-chemical system adapted to a fairly uniform environment. Although buffered to a certain extent against changes in reaction of the surrounding medium, it cannot withstand strong acids of the concentrations used in weed sprays. Whereas the rate of absorption of dilute acid may be an exponential function of concentration[14] and therefore primarily chemical in nature, the rate of killing by more concentrated solutions depends upon three processes, diffusion of hydrogen ions through the cell walls, absorption of these ions by the cell-wall material, and reaction with the living protoplasm. The relative importance of these can be only surmised, since they cannot be well differentiated in the experiments with living tissues. They will be further considered as additional data are presented.

Before discussing the experimental work with plant materials in acid solution, we might well consider briefly the various terms used to designate acidity. Normality is a measure of the titratable acid in a solution. Hydrogen-ion concentration is a measure of the equilibrium number of hydrogen ions in a solution, and is directly related to the dissociation of the solute. Until recently pH has been defined as the logarithm of the reciprocal of the hydrogen-ion concentration of a solution and as such could be computed from the dissociation of the solution as determined

from conductivity. According to modern theory, pH is defined as the logarithm of the reciprocal of the hydrogen-ion activity of a solution. Since activity is a function of free energy, pH is now computed from E.M.F. data and is usually determined by means of a hydrogen electrode or similar equipment.

In describing the killing of plant tissue with acids, use of the pH function allows a compact and accurate presentation of data. Though values computed from conductivity measurements are not accurate, the errors introduced by their use are probably no greater than those of the
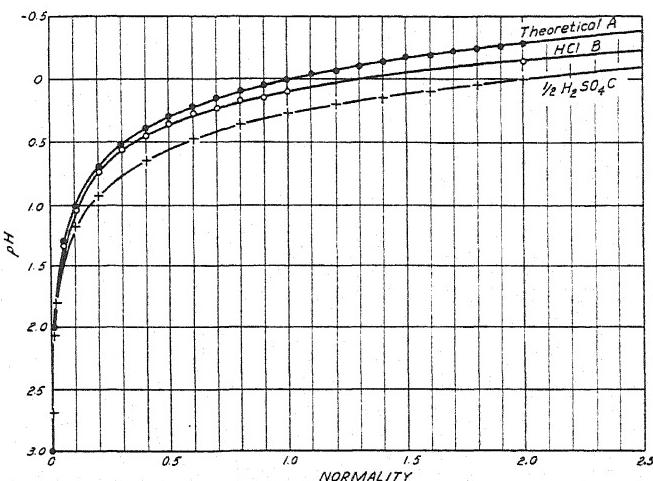
Fig. 2. The relation between normality and hydrogen-ion concentration in strong acid solutions.

determination of killing rate. Figure 2 presents curves computed from the data on hydrochloric and sulfuric acids in tables XIX and XX of Van Nostrand's Chemical Annual.[10] Curve *A* is the theoretical hydrogen-ion concentration at 100 per cent dissociation; curve *B*, the apparent concentration of hydrogen ions in hydrochloric acid solutions; and curve *C*, the values for $\frac{1}{2}$ H_2SO_4.

In order to study more accurately increasing concentrations of acid as affecting the time rate of plant-tissue killing, a series of tests were made with *Elodea* leaves and leaf sections of wild morning-glory and grass. In the first test (December, 1931), thin sections of morning-glory leaf were placed in a series of solutions, and the time of killing, as denoted by the color shift of chlorophyll, was taken. The data on this test are presented graphically in figure 3 (curve *A*). The first four solutions were dilutions of HCl; the remaining fifteen, phthalate buffer solutions. The pH values of the HCl dilutions were computed from curve *B*, figure

2. The buffer solutions were checked with a quinhydrone electrode. Since a logarithmic function of hydrogen-ion concentration is used, it was deemed best to plot these values against log time.

According to these tests the time required to kill plant tissues with strong acids depends on the hydrogen ions in the solution. When plotted
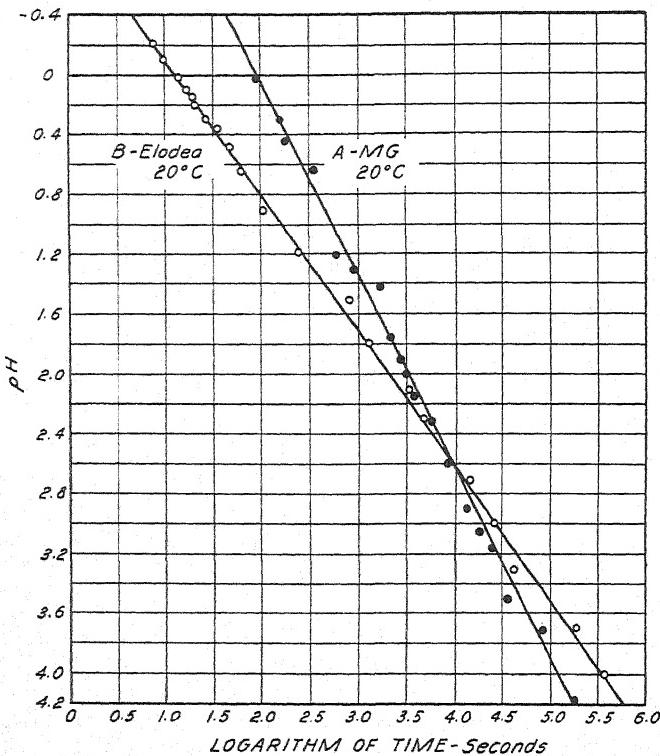
Fig. 3. The effect of hydrogen-ion concentration upon the time required for killing plant tissue with strong acids.

against the hydrogen-ion concentration computed from conductivity experiments, the data give a straight-line relation, indicating that a function related to mobility as well as activity, plays a primary part in the killing process. Though pH may not be a strictly correct symbol for this co-log function of the hydrogen ions, a new phraseology would only cause confusion. The term will be used therefore with the understanding that it does not apply strictly to the activity function.

Further tests were run, with *Elodea* leaves as indicator material. Leaves of comparable age and size were selected and a large number were pulled from the stems and stored in fresh tap water at the beginning of each test. The acid solutions were standard dilutions of CP

H_2SO_4 with distilled water and were not buffered. A large excess of solution was used each time, so that the acid was not exhausted. Each value represents the average of ten separate readings. The solutions were kept
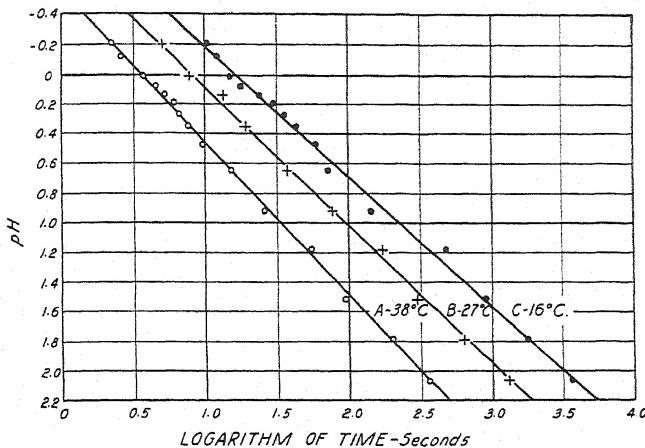
Fig. 4. The relation of temperature to rate of killing of *Elodea* leaves in sulfuric acid.
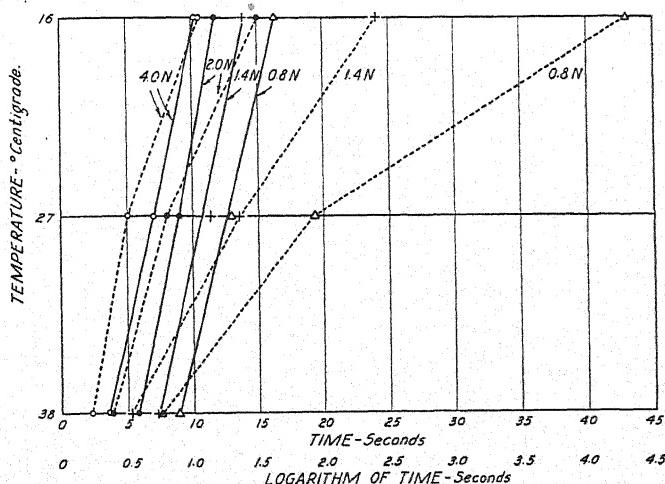
Fig. 5. Time-temperature and log time-temperature curves for killing of *Elodea* leaves in sulfuric acid. Broken lines represent time in seconds. Solid lines represent logarithm of time in seconds.

at a constant temperature, and the leaves were constantly agitated during the test. These data appear as curve *B*, figure 3. Killing in the less acid solutions is a slow process and the selection of a stage comparable with those chosen in the more concentrated solutions is attended by a considerable personal error. Furthermore the individual leaves or sections exhibit wide variations in killing rate. In spite of this, when the

values are averaged they fall close to the line as shown in figure 3. This seems to strengthen the assumption that the relation is exponential.

The effect of temperature is very apparent in the field work with acids. A temperature series was run with *Elodea* leaves. The pH-log time relations are shown in figure 4. The straight-line relation is again apparent.

The relation between temperature and time of killing for the different concentrations appears in figure 5. When temperature is plotted against log time, this relation becomes linear.
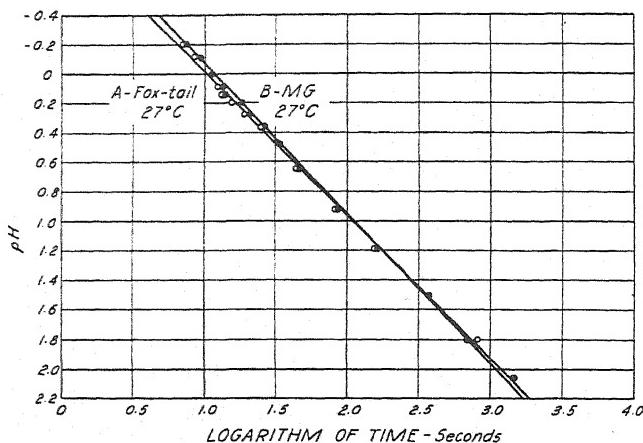
Fig. 6. The effect of hydrogen-ion concentration upon the time required to kill leaf cells of morning-glory and foxtail with sulfuric acid.

An average temperature coefficient of 2.3 has been calculated between the temperatures 17°–27° C and 27°–37°. This indicates that the chemical phases of the killing process predominate in the consumption of time. Diffusion, however, is too important a factor to be neglected. When the rates at different concentrations are considered individually, the temperature coefficients are found to decrease with increasing concentration. Possibly increased hydration of cell walls adds to the distance and hence to the time required for diffusion. Further study shows that the process is too complicated for an accurate mathematical analysis; but the importance of temperature in determining penetration of acids is clearly shown.

This method of study was applied to weeds encountered in the field by substituting leaf sections of morning-glory and foxtail for *Elodea* in the same procedure. As shown by figure 6, the results are nearly the same. The two morning-glory curves (figs. 3 and 6) differ considerably from

each other. The leaf material used in the first (fig. 3A) was taken from the field in December, 1931; that used in the latter (fig. 6B) from the greenhouse in January, 1933. In the first test the temperatures were not accurately controlled nor the times so carefully taken as in the second.

The time rate of killing plant tissue in an unlimited supply of acid has been shown, by these last experiments, to be very rapid. Whereas 0.5 N acid killed these cells in one minute or less, it has recently been found[5] that 1.0 N acid or stronger is needed to bring about satisfactory penetration in the field and that a volume of solution sufficient to wet the foliage thoroughly is required. Apparently factors other than concentration enter the problem of killing with acid. Among the most important is the effect of reaction with plant buffers.

THE PLANT BUFFER SYSTEM

The action of plant buffers was noted in some previous work on morning-glory.[4] Further studies have since been made.

The plant body is a complex organization, including among its many components organic acids and their salts, proteins and their various derivatives, basic nitrogenous compounds, colloidal carbohydrate material, and other substances, all of which may act as buffers. Preliminary experiments on the titration of plant tissue—acid mixtures with alkali showed that little or no acid is lost from the reacting mixture when tissues are treated with acid. Although some CO_2 may pass off, the addition of a quantity of alkali, equivalent to the original acid will always bring the reaction back approximately to the starting point.

The buffer capacity of ground morning-glory tissue was studied by titrating with alkali in a hydrogen cell. Ten grams of freshly ground foliage were placed with 40 cubic centimeters of 0.1 N hydrochloric acid on a water bath at 25° C. When the tissue was completely discolored, the mixture was placed in the cell of a hydrogen electrode; then, with constant mechanical stirring, the hydrogen-ion concentration was measured at 30° C as 0.1 N sodium hydroxide was added in small quantities. Figure 7 shows the titration curve along with a similar curve for pure hydrochloric acid. Curve A represents the plant titration curve, B the titration of 40 cc of 0.1 N hydrochloric acid, to which were added 10 cc of water, with 0.1 N sodium hydroxide. The ground plant tissue had an initial pH of 5.87 and a dry weight percentage of 22.2.

This ground plant tissue had an appreciable buffering capacity; only when the medium surrounding the cells differs considerably from that of the tissue itself does death occur. Although the reaction returned to neutrality when an equivalent amount of alkali was added, 23.5 cc suf-

ficed to take it back to pH 4.0, approximately the point of lethal action. Therefore, 16.0 cc carried it on to the neutral point; and, presumably, a like amount of acid would take it back to pH 4.0 again. This, then, is a measure of the effective buffer capacity of ground morning-glory tissue against the acid solutions used. Since the initial pH of the plant tissue was 5.8, the difference in titration between this and 4.0 represents the acid consumed in killing the tissue. For 10 grams of tissue, 12.5 cc of 0.1 N acid were required. This fact accounts for the large volume of acid required in treating the mass of tissue on a given area of land.
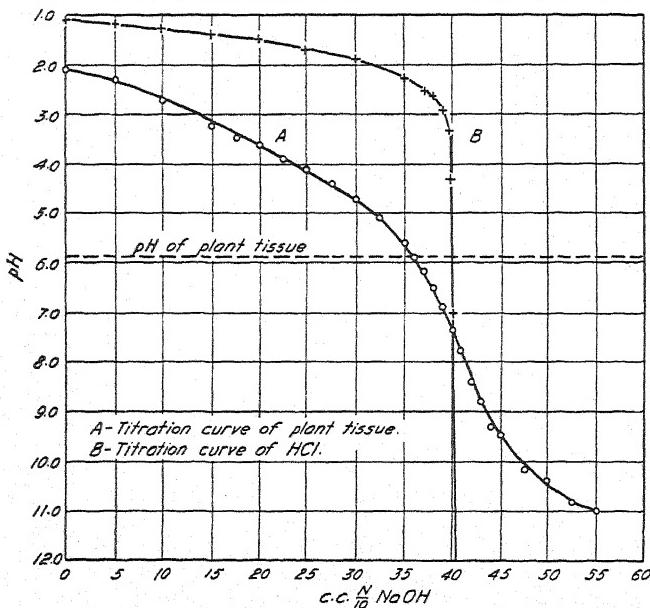
Fig. 7. Titration curves for ground morning-glory tissue and pure HCl.

THE EFFECT OF THE CUTICLE UPON PENETRATION

The other important factor in the rate of killing is the effect of the plant cuticle upon diffusion. According to popular notion, the plant cuticle is an impervious layer, whose function is to prevent evaporation of water or to bring water loss entirely under the control of the stomatal mechanism. Numerous experiments have shown, however, that the cuticle on the leaves of many plants permits a fairly rapid loss of water when the stomata are closed. Rudolph[12] found that, with the air at rest, as much as one-third of the normal water loss is cuticular. Using acids and plant poisons to study penetration of the cuticle by solutes, he found ready movement of these reagents into the leaves. The time re-

quired to kill leaves of different species with sulfuric acid varied greatly and, generally speaking, the leaves having the thicker cuticle require the longer time. All the leaves that he tried, however, were killed after some time, and his experiments as well as many of the author's show that hydrogen ions can diffuse through the leaf-cuticle of many common weeds. The rate is somewhat lower than for *Elodea* or for sectioned material.

Experiments on the rate of penetration of sulfuric acid into morning-glory leaves of different ages have shown that in general the younger leaves, even if their cuticle is thinner, are less pervious than the older ones. Because their waxy surface is so difficult to wet, intimate contact is not obtained. Thus the quality of the surface layer as well as its thickness enters the problem. Under California conditions, the older leaves of many weeds are attacked by red spiders; and these have often been observed to die more quickly than uninjured leaves. There are, apparently, two reasons: namely, puncture of the cuticle, which allows ready entry of the acid; and accumulation, by the webs and débris of the insects, of more acid per unit area than occurs on healthy leaves. So long as the leaves are not dead and dry, this insect injury apparently enhances the penetration and effectiveness of the acid sprays. Only when the live area available for treatment is considerably reduced are the effects of the application inhibited.

Another popular notion is that sprays of this nature enter the leaves through the stomata. Rudolph found no stomatal penetration in his experiments, and many tests have shown that it does not occur under normal conditions. Considering the surface tension of aqueous solutions, we see that a great pressure difference would be required to infiltrate leaves with an acid spray. Since the stomatal cavities do not become injected after spraying, and since the acid injury appears over large areas, irrespective of the presence or distribution of stomata, this action apparently does not occur.

Åslander thought that his histolological studies indicated stomatal penetration since "destruction of the cells" was "first noticeable in the neighborhood of these openings." But though the guard cells may be more easily penetrated by the acid and so more rapidly killed, this fact does not indicate that the acid actually entered the leaf through the stomata. As Åslander states further on, "It also seems to penetrate through the epidermal cells." Only if the surface tension of the solution in contact with the cuticle were greatly lowered could the solution possibly enter the stomata in any quantity. Under field conditions it probably does not enter in this manner at all.

PENETRATION OF SULFURIC ACID INTO MORNING-GLORY LEAVES

In order to apply the information thus far gained to the problem of penetration of acid solutions under field conditions, further studies have been made. These include experiments on the rate of injury of
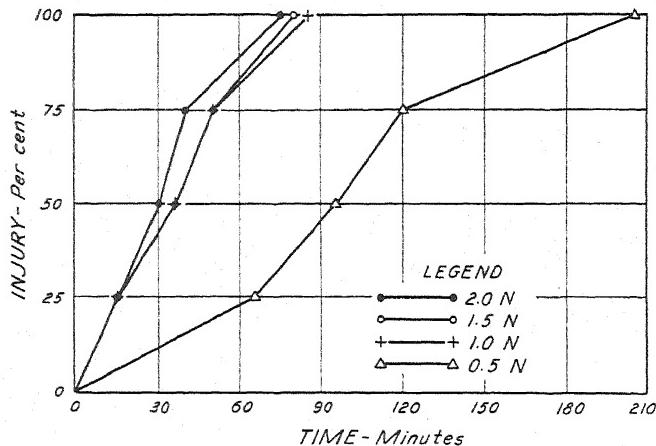
Fig. 8. The effect of immersing morning-glory leaves in sulfuric acid solutions of different concentrations.
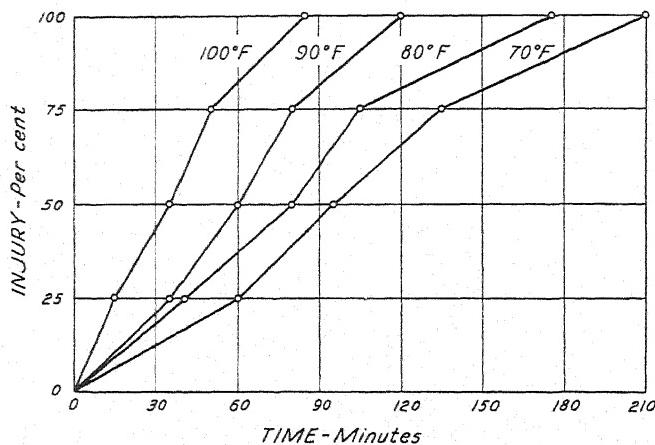
Fig. 9. The relation of temperature to injury time in sulfuric acid treatment.

morning-glory shoots immersed in acid baths of varying concentration, and studies on the rate and type of injury developing on shoots that have been dipped in the various acid solutions and subsequently exposed to the air with their cut ends in tap water. Figure 8, presenting the results at 38° C in graphic form, serves to emphasize the difference

between 0.5 N sulfuric acid and the three higher concentrations with respect to time required for a given amount of injury.

These tests were run at a series of temperatures including 21.3°, 27.0°, 32.3°, and 38° C, corresponding closely to 70°, 80°, 90°, and 100° F,
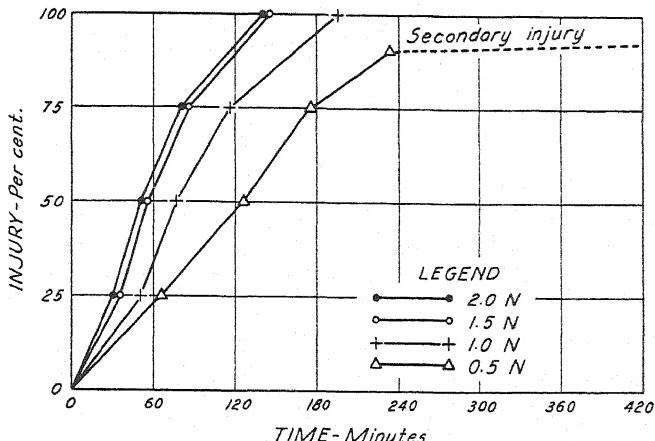
Fig. 10. Results of dipping experiments with morning-glory shoots. Injury to dipped shoots in open air.
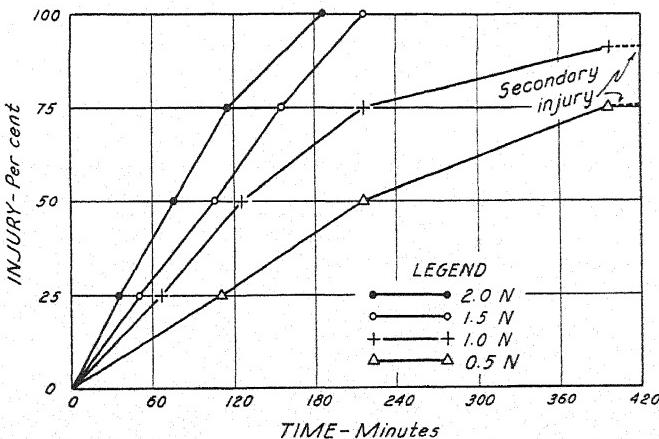
Fig. 11. Results of dipping experiments with morning-glory shoots. Injury to dipped shoots in saturated atmosphere.

within which range most sprays are applied at Davis. Since the temperature series are almost parallel throughout, only one set is presented (fig. 9). This is typical of all of the results and shows the effect of higher temperatures in accelerating the acid injury.

The effects of dipping shoots in these different acid solutions and then allowing the injury to develop as the shoots stand with their cut ends in water are more readily comparable with those obtained on sprayed

plants in the field. Figure 10 represents such a series. The slower rate of injury shows how plant buffers reduce the acid concentration as injury takes place. Evaporation tends to counteract this effect. In order to separate these two factors a separate series was run in a saturated atmosphere; the data are presented in figure 11.

Comparing figures 10 and 8, we see the influence of plant buffers in reducing the amount of acid available. As the lower concentrations in the former case were not present in sufficient amount to cause complete injury, the shoots remained mottled and finally died from secondary effects. Comparing figures 10 and 11, we see the effect of evaporation upon rate of injury. In the higher concentrations this is less noticeable than in the lower, where it is pronounced. The mottled partial killing with 0.5 N acid is very evident in the field and does not give satisfactory results.[5] The ideal situation is to have practically a saturated atmosphere after spraying and to use a sufficiently concentrated acid solution to cause rapid killing. These tests show that at least 1.0 N acid is required.

QUANTITATIVE RELATIONS

Since, with the solutions of lower acid concentration, the absolute quantity of acid available for reaction evidently becomes limiting, the quantitative phases of the problem must be considered in some detail. The growth of perennial weeds in the field varies between wide limits, so that each infestation must be treated as a separate problem. For a study of this sort it seems best to work near the upper limits. Square-yard plots were located in a dense area of morning-glory, and the top growth was removed by clipping the stems at the ground level. The foliage, rolled up, was taken to the laboratory. There each roll was weighed; sprayed thoroughly with water, by means of a knapsack sprayer, the same type of application being used as in field-plot tests; rolled up again; and reweighed. The data appear in table 1.

In spraying this material, an attempt was made to apply as much water as possible with no loss by run-off. The plots represented stands varying from a minimum growth giving complete coverage (plot 5) to a dense heavy mass (plots having 1,000 grams or more per square yard). Obviously, the variation in coverage was great; a similar variation exists under conditions of spraying in the field. The problem, therefore, is to determine the minimum quantity of solution needed for satisfactory coverage, and to relate the acid concentration to this amount so that under all conditions the entire top growth will be quickly killed.

If the plots in table 1 represent the normal variation under California conditions for stands completely covering the soil, then the average

square yard would have a cover weighing about 1.2 kilograms or 36 kilograms per square rod. In order to kill morning-glory tissue completely, the pH must be shifted from pH 5.87 to pH 4.00 (fig. 7). There would be required 36.2 minus 23.6 or 12.6 cc of 0.1 N sulfuric acid per 10 grams. This would be 126 cc of 1.0 N acid per kilogram and 36 × 126 ÷ 1,000 or 4.5 liters per square rod of foliage. The average applica-

TABLE 1

SPRAY MEASUREMENTS ON MORNING-GLORY PLOTS

Plot No.	Fresh weight, grams	Sprayed weight, grams	Water applied, grams	Volume per square rod, gallons	Volume per acre, gallons
1	1,140	1,350	210	1.7	269
2	1,960	2,350	390	3.1	499
3	1,680	2,000	320	2.6	409
4	820	1,115	295	2.4	378
5	465	675	210	1.7	269
6	725	1,010	285	2.3	365
7	1,305	1,815	510	4.1	653
8	1,130	1,575	445	3.6	570
9	1,225	1,660	435	3.5	557
10	1,150	1,650	500	4.0	640
11	1,300	1,660	360	2.9	461
12	800	1,190	310	2.5	397
13	1,230	1,530	300	2.4	384
14	1,780	2,300	520	4.2	665
15	1,085	1,400	315	2.5	404
16	1,145	1,520	375	3.0	480
17	1,045	1,415	370	3.0	474
18	1,210	1,580	370	3.0	474
Total	21,275	27,795	6,520	....	....
Average	*1,182*	*1,544*	*362.2*	*2.9*	*464*

tion as indicated in table 1 would be 2.9 gallons per square rod. After thorough trials in the field, a rate of 3.0 gallons per square rod has been selected as a standard application in plot work; it seems to provide maximum coverage for all but exceedingly heavy stands. This would be 3.785 × 3 or 11.355 liters per square rod. The normality of acid required for killing the foliage of the average plot would therefore be 4.5 ÷ 11.355 = 0.4 N; and in the ideal case where the acid can come directly in contact with all the tissue, the time required for killing would, according to figure 6, be 27.8 hours.

Similar calculations show the time required by acids of different normalities. With 0.5 N acid, for instance, there would be required 3.785 ÷ 36 × (3 × 0.5 × 1,000) = 157 cc of 1.0 N acid per kilogram, or 15.7 cc of 0.1 N acid per 10 grams. Then from figure 7, 40 — (15.7 + 3.8) = 20.5 cc, which corresponds to pH 3.7. Checking back on figure 6, 13.9
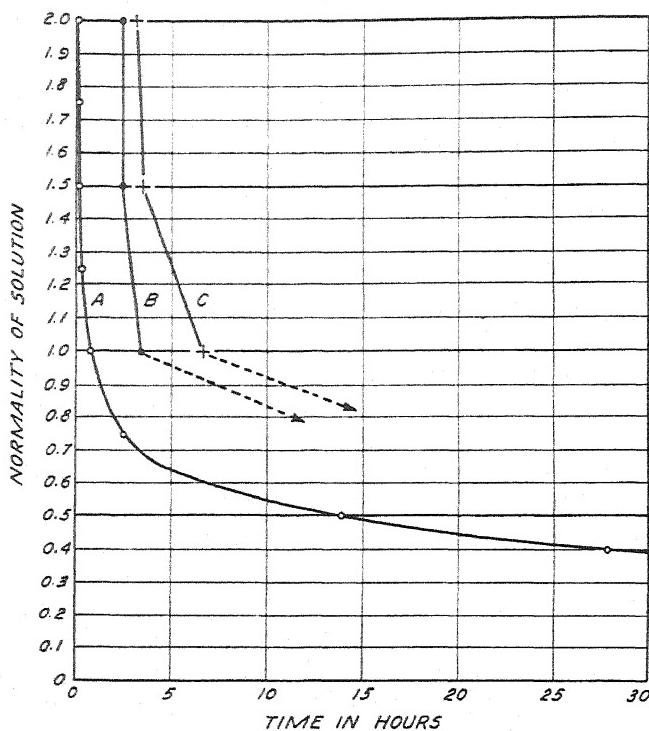
Fig. 12. The relation between normality of acid and killing time
for morning-glory tissue in sulfuric acid.

TABLE 2

Time Required for Killing Morning-Glory
Leaf Tissue with Acid of Different
Normalities

Normality	pH attained	Time required. hours
0.40	4.00	27.80
0.50	3.70	13.92
0.75	2.95	2.49
1.00	2.33	0.61
1.25	1.96	0.26
1.50	1.70	0.15
1.75	1.50	0.10
2.00	1.40	0.08

hours would be required for killing at pH 3.7. By this same method a series of calculations have been made indicating the time required for killing morning-glory tissue with different normalities of acid. These figures are given in table 2 and are represented by curve *A* in figure 12. Since the times recorded in table 2 are taken from figure 6, they apply only to the ideal case where an excess of acid is in intimate contact with thin leaf sections. From figure 10 curve *B* is drawn, which shows, in comparison with *A*, the retardation caused by the plant cuticle, and the absorption by buffers in the cell walls. Curve *C* comes from figure 11 and illustrates the part played by evaporation. High humidity in this case lengthens the time necessary for killing. Field observations often show that sprays applied on a dewy night act more slowly because the acid is not concentrated by evaporation. The broken lines in these curves represent simply the tendency of the action. Final killing in these cases was from secondary effects.

Complete killing in the dipping experiments or on sprayed plots with a low concentration of acid is difficult to obtain primarily because of uneven distribution. The solution accumulates in drops in certain regions and remains only as a very thin layer in others. The portions that retain the greater amount of the solution are completely killed, but those intervening are not and will die only when their water supply fails from disruption of the conducting system.

These studies show that for rapid and complete killing of the foliage, a certain minimum quantity of acid must be used. Under average field conditions in California 3 gallons of 1.0 N sulfuric acid per square rod has proved a satisfactory minimum. This agrees well with the data in table 1. Where foliage is exceedingly heavy, the amount might be increased considerably. Since the active hydrogen ions in the solution are taken up by the plant buffers and effectively removed from the field of action, obviously this minimum quantity cannot be reduced without some loss in effectiveness.

BUFFER EFFECT OF THE ARSENIC COMPOUNDS IN THE SOLUTION

Since the arsenious ions used in this spray solution can form undissociated weak acid molecules, their buffer action upon the mixture should be studied. The dye "gentian violet improved" of the Coleman & Bell Company was found to give color differences throughout the necessary range of acid concentration, and to be unimpaired by the addition of the arsenic solution. Since the color of this indicator in strong acid solution fades rapidly, check series of acid solutions were used in each experi-

ment, and the arsenic-acid mixtures were compared rapidly with these. The hydrogen-ion concentrations of the standard acid solutions were determined from figure 2; those of the mixtures by color comparison with the standards. Figure 13 presents the data from these tests. When the acid arsenical solution contains $\frac{M}{20}$ As$_2$O$_3$ or less and sulfuric acid 1.0 N or higher in concentration, the decrease in hydrogen ions appears negligible. According to tests already described,[5] this is the maximum concentration of arsenic needed. The buffering action of the arsenic is therefore not of primary importance in the action of this type of spray.
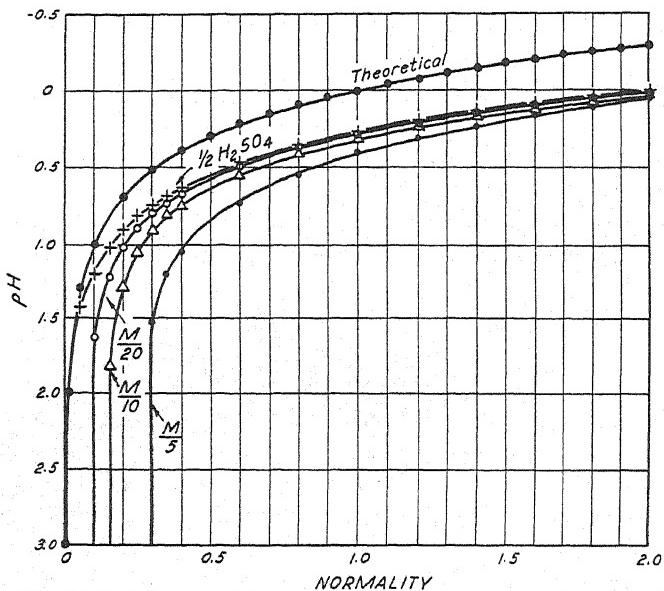
Fig. 13. Relation of pH to normality in acid arsenic solutions.
M = Mols of As$_2$O$_3$ per liter.

CORROSIVE ACTION OF THE ACID REDUCED BY ARSENIOUS IONS

The arsenious ions in the solution do have one important function besides the killing of plant tissues. Sulfuric acid is notably corrosive to metal equipment. Although the Arizona workers used oil and grease to eliminate this trouble, their method is not entirely satisfactory; and losses of machinery largely explain the limited use of this effective chemical in weed control. The addition of a little sodium arsenite greatly checks this corrosive action. Table 3 presents some data on the effect of acid solutions, with and without arsenious ions, upon iron. Small pieces of iron rod were immersed in acid solutions with and without the addi-

tion of sodium arsenite. Obviously even the smallest addition of this chemical almost stopped the action, as has been noted when the acid arsenical solution was used in commercial spray equipment. The solu-

TABLE 3

EFFECT OF SULFURIC ACID AND SULFURIC ACID–SODIUM ARSENITE MIXTURES
ON IRON*

(Weight in grams)

Solutions		Initial weight, Feb. 14	Weight on Feb. 19	Weight on Feb. 24	Weight on March 1	Weight on March 6	Weight on March 11	Weight on March 16
Acid normality	As$_2$O$_3$ concentration							
1.5	$\frac{M}{20}$	1.0408	1.0380	1.0350	1.0324	1.0295	1.0272	1.0244
1.5	$\frac{M}{40}$	1.0580	1.0550	1.0516	1.0492	1.0461	1.0444	1.0417
1.5	$\frac{M}{80}$	1.0983	1.0950	1.0912	1.0890	1.0860	1.0842	1.0818
1.5	....	1.0453	0.5914	0.1504	0.0130	....	....	....
1.0	$\frac{M}{20}$	1.0513	1.0480	1.0442	1.0420	1.0394	1.0370	1.0342
1.0	$\frac{M}{40}$	1.0872	1.0836	1.0804	1.0788	1.0758	1.0740	1.0713
1.0	$\frac{M}{80}$	1.0346	1.0310	1.0274	1.0248	1.0218	1.0197	1.0168
1.0	....	1.0796	0.7026	0.2426	0.0400	....	....	....
0.5	$\frac{M}{20}$	1.1016	1.0982	1.0940	1.0910	1.0876	1.0846	1.0815
0.5	$\frac{M}{40}$	1.0216	1.0172	1.0136	1.0110	1.0079	1.0053	1.0017
0.5	$\frac{M}{80}$	1.0022	0.9978	0.9932	0.9900	0.9870	0.9841	0.9807
0.5	....	1.0874	0.8142	0.4930	0.2834	0.1435	0.0736	....

* Iron rods immersed in solutions.

tion may be used in large quantities with little or no injury to iron, bronze, or brass equipment. It will, however, rapidly etch and dissolve the porcelain lining from the cylinders of a spray pump and ruin it for orchard spraying. All pumps used should have bronze-lined cylinders.

DISCUSSION

The most obvious effect upon living protoplasm of solutions of high hydrogen-ion concentration is killing, evidenced by cessation of streaming, by swelling, and by complete loss of the property of semipermeability. Sulfuric acid is incorporated in the acid arsenical solution under consideration for the express purpose of killing the protoplasm and rendering it permeable.

The experiments described indicate that the killing action is directly related to the hydrogen-ion concentration of the solution. The temperature coefficient suggests that the reaction is preponderantly chemical. The principal retarding factors are diffusion through the cell walls and reaction with cell wall buffers.

Although the diffusion distance may be slightly increased by swelling of the walls with concentrated acid, the rate of diffusion is enhanced by the greater concentration gradient. Apparently, therefore, the most effective means of lowering the time of penetration is to increase the acid concentration. Likewise, the amount of acid available for the killing reaction after reduction by cell wall buffers depends, since the total volume applied is limited, upon the concentration. The rate of killing and arsenic penetration is therefore determined primarily by the concentration of the acid in the spray solution.

For mechanical and economic reasons, however, the acid concentration is limited. Experiments previously cited[5] indicate that an acid concentration of 1.0 N is optimum and that 1.5 N is of maximum effectiveness under ordinary conditions. The selection of the proper concentration under any particular set of conditions, however, depends upon several factors; and, though the values given may be generally applicable, even higher concentrations may be advised under special circumstances.

Evaporation is another important factor in the action of this type of spray. From the standpoint of the translocation mechanism, low evaporation resulting in a maximum available volume would be most effective. High evaporation, however, concentrates the solution, increasing penetration rate. Probably an intermediate value would be most practical. According to field observations, killing of the foliage should be evident within a half hour after the application and should be fairly complete after two hours. Translocation, though often very rapid,[4] is enhanced by uniform and thorough penetration. Only under limited conditions is the resultant of these several reactions most favorable for rapid and complete killing of the foliage and for thorough distribution of arsenic within the root system.

The exact relations between temperature, humidity, air velocity, and concentration of the spray solution are yet to be worked out. Eventually, it is hoped these will be known so accurately that the effectiveness of a treatment may be predicted. In the meantime, these variables must be appraised by the operator and properly considered in mixing and applying the spray solution. There is a wide latitude for adjustment of the acid concentration to fit conditions and only when this technique is mastered will the optimum results be obtained.

The acid arsenical solution discussed in these pages stands alone among the weed sprays that may be prepared from commercial chemicals generally available. Its particular value depends upon its ability to penetrate foliage tissues rapidly and, by utilizing the peculiar mechanical situation that develops within the plant, to be carried deep into the roots. The sulfuric acid in the solution is of fundamental importance in the process.

For several reasons sulfuric acid is adapted for its use in this method. It is nonvolatile at field temperatures, hygroscopic, nonoxidizing, inexpensive, and readily available. Arsenic trioxide is also cheap and convenient. At present market prices the spray solution can be made for as little as one cent per gallon and in large quantities should cost even less.

Although arsenic acid has been used as a herbicide,[7, 9] laboratory and field trials have shown its inferiority to the trivalent form. It is only one-half as toxic in equimolecular concentration, more difficult to handle under field conditions, and more expensive. Although it forms an acid solution at the concentration used for application, and although the commercial product often contains a contamination of nitric acid, the total acid content at field strength (for the arsenic) is not optimum. It has not compared favorably with the trivalent form in field-plot tests.

Two years of plot tests and field trials indicate that the simple formula previously described[5] leaves little room for improvement. Future experimentation will be directed toward improving the methods of application.

SUMMARY

In *Elodea* cells killed with strong sulfuric acid, protoplasmic streaming slows and ceases; the protoplasm becomes viscous, somewhat swelled, and completely permeable; and the chlorophyll changes to a light yellowish green. The chemical reaction resulting in death of the cell takes place very rapidly in strong acid.

There exists a straight-line relation between the time rate of killing plant cells and the hydrogen-ion concentration of the solution bathing them.

Ground foliage of morning-glory has a fairly large buffering capacity. It required 12.5 cc of 0.1 N HCl to shift 10 grams of this material from the initial pH of 5.8 to pH 4.0.

The cuticles of many leaves are fairly permeable to water vapor and molecularly dissolved solutes. Sulfuric acid readily diffused into morning-glory leaves. The stomata take little or no part in the penetration of acid sprays.

Morning-glory leaves immersed in sulfuric acid solutions of 1.0, 1.5, and 2.0 normal concentrations were killed in about the same time at

100° F. Leaves in 0.5 N acid were killed much more slowly. Lower temperatures also decreased the rate of killing.

Morning-glory shoots dipped in similar acid solutions and allowed to stand with their cut ends in tap water were killed at considerably lower rates. Those dipped in 0.5 N acid were injured somewhat less rapidly than the comparable immersed shoots and were never completely killed, because of the uneven distribution of the acid. Maintaining a saturated atmosphere around these shoots lowered the killing rate, especially for the lower acid concentrations.

Under ideal conditions of contact between spray solution and the plant, application of 3 gallons of 0.5 N acid per square rod would result in an average pH of 3.7 in the tissue, and killing would require 13.9 hours. For 1.0 N acid the pH would be 2.33 and the time about 35 minutes.

Under actual field conditions, application of 0.5 N acid at a rate of 3 gallons per square rod seldom completely kills the foliage of morning-glory. Stems and basal leaves remain spotted or uninjured.

At the recommended concentrations of acid and arsenic the latter has little buffering action upon the spray solution. It practically eliminates, however, the corrosive action of the acid upon iron equipment.

The acid arsenical solution etches and slowly dissolves porcelain. Spray pumps should be equipped with brass or bronze-lined cylinders.

There remains to be studied the relation between humidity, temperature, and air velocity as affecting evaporation after the spray is applied. An accurate determination of the effects of these should increase the probability of successful use of this method.

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PRINCIPLES GOVERNING THE RECLAMATION OF ALKALI SOILS[1,2]

W. P. KELLEY[3] AND S. M. BROWN[4]

INTRODUCTION

OUR PRESENT UNDERSTANDING of alkali soils is based on two lines of investigation. Hilgard[14] was chiefly responsible for the first of these. He showed that the peculiar properties of alkali soils are caused by excessive concentrations of soluble salts; and that while high concentrations of soluble calcium, magnesium, and potassium salts are often found, sodium salts usually predominate. The other phase of the subject, developed during the past twenty years by a considerable number of investigators, is concerned with the principles of base exchange. It has been found that soluble sodium salts, upon accumulating in the soil, react by base exchange with certain constituents of the soil, thus altering the ratio of its replaceable bases. The importance of this discovery inheres in the fact that the replacement by sodium of the bases which normally occur in soils profoundly affects the chemical, physical, and crop-producing powers of the soil. Generally speaking calcium is the dominant replaceable base of normal soils. Alkali soils, on the other hand, often contain much replaceable sodium. This fact has a very important bearing on the reclamation of alkali soils.

Hilgard's View.—As is well known, Hilgard[5] devoted extended study to the origin and mode of accumulation of the soluble salts in soils, to

the tolerance of plants for the soluble salts that occur in alkali soils, and to methods of alkali-soil reclamation. He found that the soluble salts are chiefly chlorides, sulfates, carbonates, and bicarbonates of sodium, and that these salts arise, either directly or indirectly, through the natural weathering process to which rock masses are subjected in the state of nature. The ratios of the different salts vary widely from place to place, and the effects produced on plants by different salts also vary greatly. Hilgard found that Na_2CO_3 is extremely toxic, $NaCl$ somewhat less so, and Na_2SO_4 still less toxic. Hence special emphasis was placed on the soluble anions of alkali soils.

When the soluble salts have been removed, as by leaching, Hilgard believed that an alkali soil would be restored to a normal state. He recognized, however, that the physical properties of alkali soils are sometimes extremely adverse. This was attributed to the deflocculating effect of Na_2CO_3. If Na_2CO_3 is present, he held that it may be necessary to apply gypsum, or some substance which produces similar effects, before it is possible to leach out the soluble salts. Hilgard assumed that the chemical effect of gypsum would be that of the well-known chemical reaction which takes place when a soluble calcium salt is added to a solution of Na_2CO_3, as follows:

$$CaSO_4 + Na_2CO_3 \rightleftarrows CaCO_3 + Na_2SO_4.$$
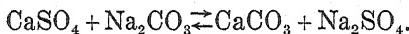
Thus upon applying gypsum the alkaline and therefore deflocculating Na_2CO_3 will be converted into insoluble $CaCO_3$ and neutral Na_2SO_4; consequently, the soil will become flocculated and then the soluble salts can be effectively leached out.

Hilgard subdivided alkali soils into two classes, namely, "white alkali" and "black alkali." The soluble salts of the former are neutral compounds, chiefly chlorides and sulfates, while the latter contain Na_2CO_3 and may also contain high concentrations of $NaCl$ and Na_2SO_4.

Present View.—Consideration of the researches of de Mondesir,[21] Hissink,[15] Gedroiz,[7] de 'Sigmond,[24] de Dominicis,[6] Cummins and Kelley,[5, 18] and other students of base exchange, shows that there is an important deficiency in Hilgard's views. He failed to take into consideration the extremely important fact that under certain conditions the soluble sodium salts react by base exchange with the clay and humus of the soil. The Na-clay and Na-humate thus formed, being insoluble in water, are not readily removed from the soil by leaching. Moreover, it is now known that Na-clay and Na-humate cause special difficulties in the practical reclamation of alkali soil. Hence the mere removal of the soluble salts will not necessarily restore the soil to a normal condition.

It is well established that soil containing replaceable sodium tends

to become highly deflocculated and more or less alkaline upon leaching, owing to hydrolysis of Na-clay and Na-humate.[5, 7, 8, 11, 21, 22] The soluble product of the hydrolysis, NaOH, becomes converted into Na_2CO_3 and $NaHCO_3$ by CO_2. When the concentration of soluble sodium salts has been reduced to a low level, interaction sets in between Na-clay or Na-humate and $CaCO_3$, which also results in the formation of Na_2CO_3. Thus the soil solution of an alkali soil will tend to be alkaline in a chemical sense as long as sodium constitutes a high percentage of the total replaceable cations.

Gedroiz,[7, 8] Hissink,[15] de 'Sigmond,[24] and others [5, 18] have found that base exchange has an important effect on the physical properties of the soil. According to Gedroiz,[10] the pronounced deflocculation that develops upon leaching Na-saturated soil is due to the OH ions that are formed through hydrolysis. He concluded that the consequence of this deflocculation is the gradual development of peculiar morphological structures within the soil profile. Since leaching a sodium saline soil promotes the formation of Na_2CO_3 and produces adverse physical effects on the soil, Gedroiz[7, 8] concluded that leaching may produce actual injury rather than benefit to the soil.

Relation of Black Alkali and White Alkali to Solonetz and Solonchak.—For many decades Russian investigators have subdivided alkali soils into two classes, namely, *solonetz* and *solonchak*. These two terms are now widely used in the soil literature of the world, but not always in precisely the sense of the original Russian usage. Strictly speaking, solonetz denotes an alkali soil which contains a relatively low content of soluble salts. However, Glinka[12] and other soil morphologists in Russia, and more recently students of soil classification in the United States, have used the term solonetz to denote certain morphological features of the soil profile, but without special regard either to the soluble salt content or the specific base that is held by the exchange complex of the soil. On the other hand, Gedroiz[8, 11] employed the term solonetz to denote soil containing replaceable (absorbed) sodium.

Solonchak denotes a soil high in soluble salts. Generally speaking white-alkali soils belong to the solonchak class, but solonchak may contain Na_2CO_3 as well as chlorides and sulfates. The profile of solonchak does not show characteristic morphological structures. The solonchaks are subdivided into sodium solonchaks, calcium solonchaks, chloride solonchaks, sulfate solonchaks, etc., depending upon the specific kind of salt that predominates.

Gedroiz showed that solonetz is derived from solonchak through leaching, but that only one kind of solonchak can be converted into solonetz, namely, sodium solonchak. Calcium solonchak does not pass into solonetz

upon leaching because the formation of Na-clay does not take place under these conditions. Although Gedroiz[8, 11] pointed out that sodium solonchak must necessarily contain more or less replaceable (absorbed) sodium, his usage of the term solonetz in various places in his publications is, as pointed out above, in the sense of soil containing replaceable (absorbed) sodium. Thus it is evident that Gedroiz has placed special emphasis on the cations of alkali soils, whereas Hilgard placed the emphasis on the soluble anions.

Gedroiz'[10] views as to the origin of the solonetz morphological structures are briefly as follows: Soluble sodium salts, upon accumulating in the soil, react with the base-exchange complex of the soil. This leads to the formation of simple calcium and magnesium salts and of Na-clay and Na-humates (absorbed sodium). The Na-clay and Na-humates thus formed remain in a state of flocculation as long as the soil contains a high concentration of soluble salts, but when the soluble salts have been leached out the clay constituents pass into a condition of high dispersion or deflocculation. The dispersed particles then gradually become elutriated downward, in consequence of which relatively dense subsoil horizons are formed. The zones, in which the elutriated particles accumulate, being relatively rich in Na-clay, develop the peculiar morphological structures that are characteristic of solonetz, owing to the inherent properties of Na-clay when largely freed from soluble electrolyte. Thus the solonetz morphological structures arise from sodium salines through leaching.

Solonetz is also relatively unstable chemically. Its chemical instability is due to the fact that Na-clay and Na-humate undergo hydrolysis upon leaching out the soluble salts, giving rise to H-clay and H-humate. Gedroiz holds that H-clay is itself relatively unstable and that it may gradually disintegrate into simple oxides. The pronounced development of this disintegration Gedroiz looks upon as the degradation stage of solonetz for which he employed the term *solodi*. However, if $CaCO_3$ be present in the soil, Ca ions will pass into solution upon leaching out the soluble salts and these ions will then begin to replace Na from the exchange complex with the consequent formation of Na_2CO_3 and stable Ca-clay. Hence $CaCO_3$ tends to prevent the decomposition of the clay constituents of a solonetz. Therefore, solonetz may either pass into solodi upon prolonged leaching or into a condition similar to that of normal soil.

Black-alkali soil, as defined by Hilgard, is related to solonetz but is not necessarily identical with solonetz. As stated already, the morphological structures that are characteristic of solonetz arise through the leaching of sodium salines, and it is probable that the solonetz structures

arise only in this way. As stated above, the formation of Na_2CO_3 sets in when the concentration of soluble sodium salts becomes reduced to a low level by leaching, owing to hydrolysis of Na-clay and Na-humate, or to the reaction of these constituents with $CaCO_3$. However, the occurrence of Na_2CO_3 in the soil is not necessarily proof that leaching of that particular soil has actually taken place. Sodium carbonate may be transported by means of water from one place to an entirely different location. Gedroiz[11] has pointed out that soil into which Na_2CO_3 arises by capillarity inevitably acquires the chemical property of solonetz, whether it be subjected to leaching or not. Moreover, since NaCl and Na_2SO_4 may arise into the surface soil from the subsoil by capillarity along with Na_2CO_3, black-alkali soil may have the chemical properties without the morphological structures of solonetz. Thus it follows that a sodium solonchak may contain Na_2CO_3 and therefore conform to Hilgard's definition of black-alkali soil.

Briefly, then, black alkali denotes soil which contains Na_2CO_3, while solonetz has a twofold significance, namely morphological and chemical, i.e., it refers to certain morphological features of the soil profile and to the presence of replaceable (absorbed) sodium. Since soils which contain Na_2CO_3 also probably always contain more or less replaceable (absorbed) sodium, black alkali is equivalent to solonetz in a chemical sense, but not necessarily so in a physical sense.

EXPERIMENTAL RESULTS

The results of the authors' investigations on two widely different types of alkali soil will be presented as a basis for discussion of the important principles that are involved in the reclamation of alkali soils in general.

BLACK-ALKALI SOILS

Description of the Fresno Type.—The black-alkali soil of the Fresno area of California is a fine sandy loam low in organic matter. The soil and subsoil are approximately uniform in texture to a depth of 2 to 3 feet, below which there is a compact layer 2 to 6 inches in thickness, which is rich in $CaCO_3$. Immediately below this calcareous layer, soil material is found similar to that on or near the surface of the soil, and this extends down to variable depths where another compact calcareous layer is encountered. This succession of sandy loam materials and calcareous horizons extends to a relatively great depth. The surface soil contains somewhat less than 0.1 per cent $CaCO_3$. The profile of this soil does not present a clearly defined sequence of soil horizons such as occur

in more mature soils of other localities. Moreover, the morphological structures that are characteristic of solonetz are also absent from it. This soil is therefore a sodium solonchak. The alkalinity of this soil is due primarily not to hydrolysis of Na-clay and Na-humate of the soil, but to Na_2CO_3 that has accumulated by capillary rise from subsoil horizons. This fact has some bearing on the experimental results, as will be pointed out later.

Treatments.—In 1920 a series of plot experiments was begun on an area of this soil on the Kearney Ranch near Fresno. At that time the soil was extremely toxic to cereals, alfalfa, and other crops. The soil contained a relatively high concentration of soluble sodium salts consisting chiefly of chloride, sulfate, carbonate, and bicarbonate. The total concentration of soluble salts was greatest near the surface of the soil. The clay and humus of the soil had been largely converted, through base exchange, into sodium compounds. It was expected, therefore, that leaching would cause more or less hydrolysis of the base-exchange constituents. Further reference to this point will be made later.

The discussion of the Fresno experiments will be confined to three plots, namely, plot 4, treated with 15 tons per acre of gypsum, 9 tons of which were applied in 1920 and 6 tons in 1921; plot 5, a check plot, to which no material other than irrigation water has been applied; and plot 10, which was treated in 1921 with 3,600 pounds per acre of elemental sulfur.

After applying gypsum to plot 4 this plot and also plot 5 were flooded with water, and were kept submerged by repeated applications of water, for two successive periods of approximately three weeks each in the summer of 1920 and again in 1921. The sulfur plot No. 10, was flooded for a period of about three weeks in September, 1921. Subsequently no material other than irrigation water has been applied to any of these plots. The amount and frequency of irrigation has been somewhat in excess of that usually practiced in this locality.

Effect of Gypsum and Sulfur.—The crop records are reported in table 1. It is shown that both the gypsum and the sulfur plots have produced good yields of crops for a period of several years. The data presented in tables 2 and 3 confirm those reported in 1928,[16, 19] in showing that the concentration of water-soluble salts[6] has been greatly reduced by the treatments. In fact the gypsum and sulfur plots do not now contain injurious concentrations of soluble salts at any depth above 48 inches. The data on replaceable bases reported in tables 4 and 5 show that replaceable sodium has also been chiefly removed from these plots.

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[6] The water-soluble constituents were determined in 1:5 water extracts of the soil.

TABLE 1
CROP RECORDS OF THE FRESNO EXPERIMENTS
(Pounds per acre)

Year	Crop	Plot 4; treated with gypsum, 1920	Plot 5; leached	Plot 10; treated with sulfur, 1921
1921	Barley hay	2,865	1,770	....
1922	Barley hay	3,216	2,584	300
1923	*Melilotus indica*	Plowed under as green manure	....	....
1924	*Melilotus alba*	Plowed under as green manure	....	....
1925	Alfalfa	5,955	3,585	18,467
1926	Alfalfa	Uncropped	Uncropped	23,658
1927	Alfalfa	11,742	6,255	20,138
1928	Alfalfa	18,858	15,446	5,873*
1929	Alfalfa	16,785	15,617	10,570
1930	Alfalfa	19,082	17,494	18,902
1931	....	Uncropped	Uncropped	Uncropped
1932	Cotton	1,622	1,874	....

* Barley hay.

TABLE 2
WATER-SOLUBLE SALTS, PLOT 4; TREATED WITH GYPSUM
(Milliequivalents per 100 grams)

Depth in inches	CO₃	HCO₃	Cl	SO₄	Ca	Mg	K	Na
				Before treatment (1920)				
0–12	0.94	0.80	0.32	0.20	trace	trace	0.20	2.06
12–24	0.49	0.51	0.46	0.21	trace	trace	0.20	1.47
24–36	0.43	0.34	0.39	0.14	trace	trace	0.21	1.09
36–48	0.15	0.40	0.32	0.13	trace	trace	0.18	0.82
				After treatment (1931)				
0–12	0.00	0.40	0.05	0.02	0.23	trace	trace	0.24
12–24	0.00	0.32	0.08	0.04	0.17	trace	trace	0.27
24–36	0.15	0.35	0.08	0.04	0.12	trace	trace	0.50
36–48	0.15	0.50	0.10	0.04	0.07	trace	trace	0.67

TABLE 3
WATER-SOLUBLE SALTS, PLOT 10; TREATED WITH SULFUR
(Milliequivalents per 100 grams)

Depth in inches	CO₂	HCO₃	Cl	SO₄	Ca	Mg	K	Na
				Before treatment (1921)				
0–12	1.15	0.90	1.53	0.65	trace	trace	0.17	4.06
12–24	0.60	0.64	1.24	0.41	trace	trace	0.17	2.72
24–36	0.53	0.68	0.70	0.19	trace	trace	0.20	1.90
36–48	0.42	0.57	0.50	0.12	trace	trace	0.20	1.41
				After treatment (1931)				
0–12	0.00	0.25	0.05	0.04	0.10	trace	trace	0.24
12–24	0.00	0.30	0.05	0.05	0.12	trace	trace	0.28
24–36	trace	0.50	0.07	0.07	0.07	trace	trace	0.57
36–48	0.30	0.62	0.07	0.10	trace	trace	trace	0.79

TABLE 4

REPLACEABLE BASES, PLOT 4; TREATED WITH GYPSUM

Depth in inches	Milliequivalents per 100 grams			Na as per cent of total	pH
	Ca + Mg	K	Na		
Before treatment (1920)					
0–12	1.08	0.23	3.13	70	9.67
12–24	0.42	0.98	2.87	67	9.42
24–36	1.78	0.28	2.41	54	9.59
36–48	2.57	0.34	1.59	35	9.11
After treatment (1931)					
0–12	5.05	0.00	0.27	5	7.53
12–24	4.59	0.00	0.40	8	8.10
24–36	4.63	0.00	0.43	8	8.30
36–48	4.13	0.00	1.00	19	8.70

TABLE 5

REPLACEABLE BASES, PLOT 10; TREATED WITH SULFUR

Depth in inches	Milliequivalents per 100 grams			Na as per cent of total	pH
	Ca + Mg	K	Na		
Before treatment (1921)					
0–12	1.35	0.44	2.51	58	9.67
12–24	1.21	0.34	2.90	65	9.20
24–36	3.19	0.20	2.00	37	8.98
36–48	3.61	0.13	1.26	25	9.43
After treatment (1931)					
0–12	4.06	0.29	0.21	5	7.05
12–24	3.75	0.15	0.44	10	7.50
24–36	4.05	0.37	0.38	8	8.80
36–48	3.66	0.30	0.85	17	9.30

Before the experiments were begun, sodium comprised from 60 to 70 per cent of the total replaceable bases of the soil. The treatments with sulfur and gypsum have, however, brought about the replacement of practically all the sodium by calcium. Therefore, the soil of neither of these plots can now be properly regarded as alkali soil. It has become normal in every essential particular.

Effect of Leaching.—Although good results were obtained within a comparatively brief period following the application of gypsum or sulfur, the check plots failed to produce satisfactory yields of crops for several years. In fact certain parts of the check plots remained totally unproductive for some time after these experiments were begun, notwithstanding the fact that the leaching, to which they were subjected, was effective in removing the principal part of the soluble salts. During the course of the experiment it was noted, however, that the size of the unproductive spots within the check plots diminished from year to year.
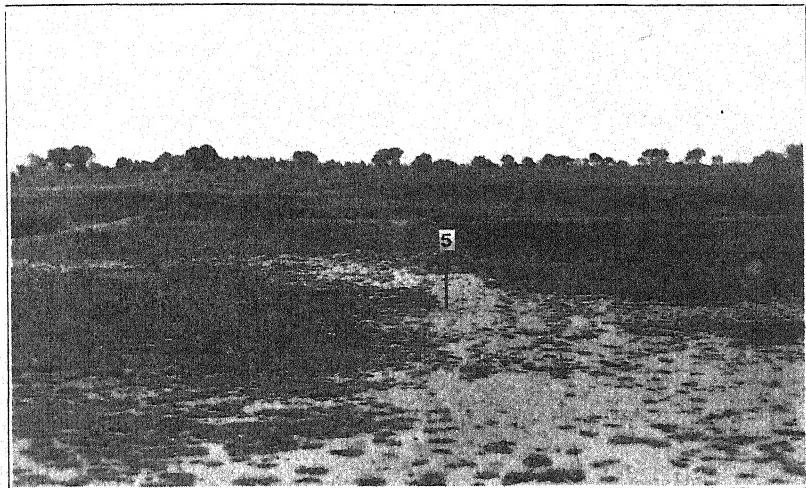
Fig. 1. Barley, plot 5 of the Fresno experiment, after it was leached in 1920. The barley crop failed to grow on much of this plot. Photographed April 20, 1921.

These spots finally disappeared entirely in 1928, when a complete stand of alfalfa was obtained over practically all parts of the check plots. Subsequently the check plots have produced large yields of crops. In 1932 cotton was planted on these plots. The cotton seeds germinated uniformly, and fully as large yields were obtained from the check plots as from the gypsum plots (table 1 and figs. 1, 2, 3).

Gedroiz,[7, 8] as stated already, concluded that the suitability for crop growth of a sodium saline soil, such as that of these experiments, would be adversely affected rather than benefited by leaching, owing to deflocculation and the excessive alkalinity that would be produced through the formation of Na_2CO_3. As is well established, the formation of Na_2CO_3 will not take place as long as the soil contains a high concentration of soluble sodium salts owing to the effect of a common ion on hydrolysis, but when the concentration is reduced sufficiently through leaching, it is reasonable to expect that more or less Na_2CO_3 would be formed in the

soil. The crop yields obtained during the early years of this experiment seemed to support Gedroiz' hypothesis, for, as pointed out already, the check plots failed to produce satisfactory growth of crops for several years (figs. 1 and 2).

Fig. 2. Alfalfa, plot 5, after having been leached in 1920 and 1921. The plants shown in the foreground are alkali weeds consisting chiefly of *Tissa salina*. When this photograph was taken (September 16, 1925) the practical failure of alfalfa shown thereon indicated that this plot had not been benefited by leaching.
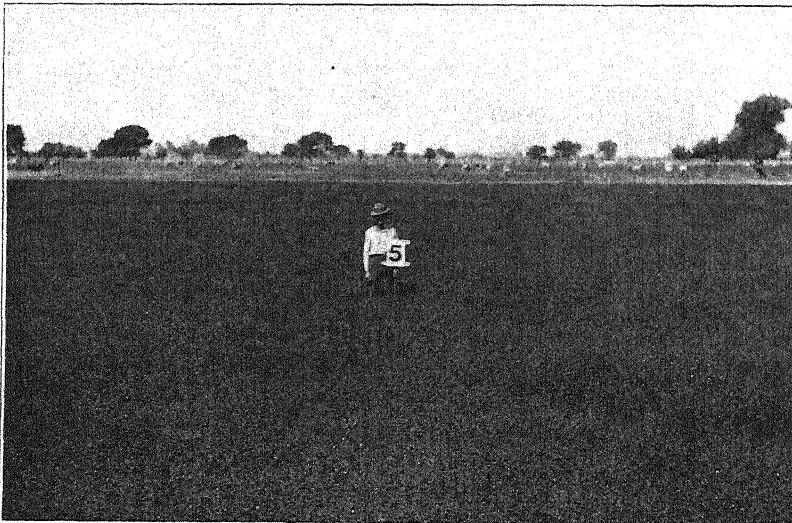
Fig. 3. Alfalfa, plot 5, photographed August 23, 1930. The normal growth of alfalfa presents a striking contrast to that of 1925 (see fig. 2). No material other than irrigation water has been applied to this plot.

The data reported in tables 6 and 7 show the transformations that have been produced by leaching. It will be noted that the water-soluble salts and the replaceable sodium have been largely removed from the upper two feet of the soil. On the other hand, the content of replaceable sodium in the subsoil (36–48 inches) of this plot has been increased slightly, probably because of the leaching down of the soluble sodium salts that originally occurred near the surface of the soil.

TABLE 6

WATER-SOLUBLE SALTS, PLOT 5, AS AFFECTED BY LEACHING

(Milliequivalents per 100 grams)

Depth in inches	CO_3	HCO_3	Cl	SO_4	Ca	Mg	K	Na
Before leaching (1920)								
0–12	1.05	0.73	0.62	0.33	trace	trace	0.14	2.59
12–24	0.49	0.52	0.62	0.26	trace	trace	0.11	1.78
24–36	0.42	0.38	0.54	0.17	trace	trace	0.24	1.27
36–48	0.15	0.38	0.34	0.10	trace	trace	trace	0.97
After leaching (1931)								
0–12	trace	0.52	0.05	0.05	0.06	trace	trace	0.56
12–24	0.15	0.45	0.07	0.06	0.04	trace	trace	0.69
24–36	0.55	0.50	0.07	0.03	trace	trace	trace	1.15
36–48	0.70	0.65	0.12	0.05	trace	trace	trace	1.52

TABLE 7

REPLACEABLE BASES, PLOT 5, AS AFFECTED BY LEACHING

Depth in inches	Milliequivalents per 100 grams			Na as per cent of total	pH
	Ca + Mg	K	Na		
Before leaching (1920)					
0–12	1.21	0.33	2.90	65	9.18
12–24	1.17	0.33	3.61	70	9.15
24–36	2.89	trace	2.49	46	8.61
36–48	3.46	0.23	1.43	28	8.59
After leaching (1931)					
0–12	4.66	0.00	0.30	6	8.07
12–24	4.44	0.00	1.20	21	8.70
24–36	4.35	0.00	1.54	26	9.03
36–48	3.07	0.00	3.45	53	9.30

Since the subsoil of the check plot still contains considerable soluble carbonate and replaceable (absorbed) sodium, it has not yet been completely restored to a state of normality. However, the data strongly indicate that a continuation of ordinary methods of soil management, provided it includes the liberal application of irrigation water, will ultimately bring about the complete elimination of sodium carbonate and replaceable sodium from the subsoil of the check plot, just as has already taken place in the gypsum and sulfur plots. Moreover, there is no evidence that this plot has been actually injured by leaching. The results, therefore, are not in harmony with Gedroiz' conclusion.

TABLE 8

COMPOSITION OF IRRIGATION WATER

(Milliequivalents per liter)

	Well water, Fresno experimental tract	Colorado River water*
HCO₃	2.54	2.99
Cl	0.42	3.20
SO₄	0.29	7.94
Ca	1.50	4.90
Mg	1.00	2.86
Na	0.80	6.44

* Mean on 41 analyses of samples taken at Yuma, Arizona, as reported in: Scofield, C. S., and L. V. Wilcox. Boron in irrigation waters. U. S. Dept. Agr. Tech. Bul. **264**: 58. 1931.

COMPOSITION OF IRRIGATION WATER

The salt content of the irrigation water is important in connection with the reclamation of alkali soil, as was pointed out previously by Scofield and Headley,[23] and by Kelley and Brown.[17] The experiments of Gedroiz,[7] de Dominicis,[6] Cummins and Kelley,[5] and others, showing that extremely adverse chemical and physical conditions develop in sodium saline soils upon leaching out the soluble salts, were all conducted with the aid of distilled water. On the other hand, irrigation waters usually contain more or less dissolved salts. Hibbard[13] showed that water containing even a relatively low concentration of dissolved salts is more effective as a leaching agent than distilled water. American irrigation waters not only contain more or less dissolved salts, but, what is still more important, they commonly contain considerable calcium salts. When an alkali soil is leached with water of this kind much less hydrolysis takes place than with distilled water; the Ca ions and Mg ions of the water tend to replace sodium from the exchange complex, and the electrolytes flocculate the soil colloids. The net result is that the soil tends to remain in a flocculated condition.

The effectiveness of irrigation water in the reclamation process is dependent both on its total content of soluble salts and on the ratio of its divalent to monovalent bases. The irrigation water used in the Fresno experiments contains about 250 p.p.m. total salts with a ratio of sodium to divalent bases of approximately 1:3 (see table 8). Many other irrigation waters are still more favorable, since they contain considerably higher concentrations of calcium and magnesium salts.

It is not possible to assign a quantitative value to the effects produced by the irrigation water that was used in the foregoing experiments, owing to the complexity of the components of the systems under consideration. However, in addition to serving as a vehicle for the removal of soluble salts, it is highly probable that the irrigation water, through its content of calcium salts has, nevertheless, contributed something towards the conversion of Na-clay into Ca-clay.

THE ROLE OF CaCO$_3$ IN THE RECLAMATION PROCESS

The high concentration of sodium salts, which had accumulated in the Fresno soil before the experiments were begun, undoubtedly brought about the replacement of a large part of the replaceable calcium and magnesium by sodium. However, the calcium and magnesium thus replaced were not converted into soluble calcium and magnesium salts, but were probably precipitated in the soil mass principally as carbonates, through combination with soluble CO$_3$ ions, and to a lesser extent as silicates. Consequently, the leaching process has not removed any important amount of calcium and magnesium from the soil. The available data justify the conclusion that, upon reducing the concentration of soluble sodium salts by leaching, the labile calcium minerals (probably chiefly CaCO$_3$) began to react with the sodium exchange complex (Na-clay and Na-humate). Consequently sodium has been gradually replaced by calcium with the formation of Na$_2$CO$_3$ and NaHCO$_3$. A possible explanation of the reactions is as follows:

$$\text{Na-clay} + \text{CaCO}_3 \rightleftarrows \text{Ca-clay} + \text{Na}_2\text{CO}_3$$
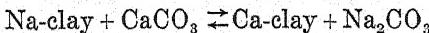
Na$_2$CO$_3$, thus formed, would be converted, partially at least, into NaHCO$_3$ by the action of CO$_2$ of the soil.

Or, it may be assumed that hydrolysis first intervened and that the H-clay thus formed reacted with CaCO$_3$ as follows:

$$\text{Na-clay} + \text{HOH} \rightleftarrows \text{NaOH} + \text{H-clay}$$
$$\text{H-clay} + \text{CaCO}_3 \rightleftarrows \text{Ca-clay} + \text{H}_2\text{CO}_3$$
$$\text{H}_2\text{CO}_3 + \text{NaOH} \rightleftarrows \text{NaHCO}_3 + \text{H}_2\text{O}$$
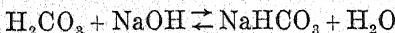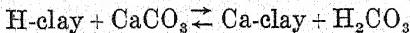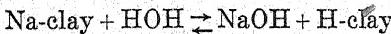
Therefore, the end products would be essentially the same whether CaCO$_3$ reacted directly with Na-clay or indirectly with H-clay formed

by hydrolysis of Na-clay. In either case these reactions would bring about the replacement of sodium and a corresponding increase in replaceable calcium. Sodium carbonate and $NaHCO_3$ formed by these reactions have been leached down into the subsoil and partially into the underdrainage.

As Cummins and Kelley[5] pointed out, the extent to which Na-clay undergoes hydrolysis in pure water is limited by two factors, namely, the low ionization of water and the concentration and composition of the soluble sodium salts that are present. Theoretically we would expect that at the equilibrium point of this reaction the ionization constant of the Na-clay would equal that of the NaOH formed. Since the former is relatively low, the total concentration of NaOH will also be low. However, CO_2, which is commonly present in soils to some extent, exerts a potent influence, first through increasing the ionization of water and secondly through converting NaOH into $NaHCO_3$. Thus carbonated water will be more effective than pure water in the conversion of Na-clay into H-clay.

That $CaCO_3$ has played the important role indicated above is shown by the determination of the insoluble CO_3 of the soil. It has been found (table 9) that the content of insoluble CO_3 has been materially altered as a result of these treatments. It will be noted that the check plot, No. 5, has lost a large part of its insoluble CO_3. It is logical to conclude, therefore, that the changes which have taken place in the replaceable bases of the check plot have been produced, partially at least, by $CaCO_3$.

TABLE 9

PERCENTAGE OF INSOLUBLE CO_3 AS AFFECTED BY VARIOUS TREATMENTS

Depth in inches	Plot 4		Plot 5		Plot 10	
	Before treatment (1920)	After treatment with gypsum (1931)	Before leaching (1920)	After leaching (1931)	Before treatment (1921)	After treatment with sulfur (1931)
0–12	0.0294	0.0372	0.0419	0.0121	0.0728	0
12–24	0.0729	0.0989	0.0562	0.0025	0.0120	0

It will also be noted that the insoluble CO_3 of the gypsum plot has been increased slightly. This was probably due to the formation of $CaCO_3$ through the interaction of $CaSO_4$ (gypsum) and soluble CO_3 of the soil. In this connection it may be pointed out that these results afford strong evidence that this soil actually contains Na_2CO_3, despite the claim of Breazeale and McGeorge[3] that black-alkali soils do not contain Na_2CO_3.

The theoretical reactions, which are expected to take place upon applying sulfur, readily account for the loss of insoluble CO_3 from the sulfur plot. As is well known, sulfur undergoes biological oxidation in soil, which leads to the formation of H_2SO_4. The acid thus formed reacts with $CaCO_3$. The following equations illustrate these reactions:

$$2\,S + 3\,O_2 + 2\,H_2O = 2\,H_2SO_4$$
$$H_2SO_4 + CaCO_3 \rightleftarrows CaSO_4 + H_2CO_3$$
$$H_2CO_3 + CaCO_3 \rightleftarrows Ca(HCO_3)_2$$
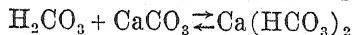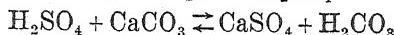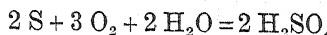
Therefore sulfur, upon oxidation,[7] converts $CaCO_3$ into $CaSO_4$ and $Ca(HCO_3)_2$. The calcium thus made soluble functions in the replacement of sodium from the exchange complex. Or it is possible that H_2SO_4 reacted with Na-clay and Na-humate, converting the same into H-clay and H-humate and Na_2SO_4, and that H-clay and H-humate then reacted with $CaCO_3$, as was discussed above.

Obviously the sulfur oxidation product (H_2SO_4) and gypsum also converted Na_2CO_3, originally present in the soil, into Na_2SO_4.

As long as an alkali soil contains a high concentration of soluble sodium salts, $CaCO_3$ is unable to bring about the replacement of sodium, owing to the high concentration of Na ions that are continually present in the soil solution. However, when the concentration of Na ions is reduced sufficiently by leaching, Ca ions furnished by the solution of $CaCO_3$ begin to replace sodium, and thus a gradual increase in replaceable calcium and a corresponding diminution in the replaceable sodium are brought about.

Dialysis Experiments.—That $CaCO_3$ has played the role indicated above has been further confirmed by dialysis experiments. Samples of soil artificially saturated with sodium and which were free from water-soluble salts, were mixed with $CaCO_3$ and then subjected to dialysis. The dialyzate was removed daily from the external chamber of the dialyzer. After the dialysis had been continued for two weeks, the soil was found to be free from replaceable sodium and its base-exchange complex was calcium saturated. Analysis showed that sodium was the chief base which diffused through the semipermeable membrane, the amount thus removed from the soil being approximately equal to the original content of replaceable sodium. Similar results were obtained with a sample of the Fresno soil and also with an alkali soil from Utah.

Electrodialysis Experiments.—Electrodialysis experiments have also been made with these soils. In these experiments the rate of the reaction between $CaCO_3$ and the sodium-saturated soil (Na-clay) was much more

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[7] These equations are intended to represent the end results of sulfur oxidation. It is probable that the oxidation passes through one or more intermediate stages before the sulfate form is reached.

rapid than that which took place in the dialysis experiments referred to above, but the final result was the same. It was found that the replaceable sodium was removed from the soil quantitatively and that calcium took its place in the exchange complex. It was necessary, of course, to discontinue the electrodialysis before the last traces of $CaCO_3$ were decomposed; otherwise the soil would probably have become base unsaturated.

The validity of the foregoing argument as to the role of $CaCO_3$ in the leaching of a sodium saline soil rests essentially on two principles: (1) The replacing power of Ca ions is considerably greater than that of Na ions. Students of base exchange generally accept this as a fact. As soon as the concentration of soluble sodium is sufficiently reduced by leaching, Ca ions furnished by $CaCO_3$ gradually effect the replacement of sodium. (2) H-clay and H-humate, whether formed by hydrolysis or by the action of acids, react actively with $CaCO_3$. The result is the replacement of H ions by Ca ions and the consequent augmentation of replaceable calcium. Generally speaking, it is well agreed that this is the most important reaction which takes place when acid soils are limed.

The importance of $CaCO_3$ in the process of alkali soil reclamation has been strikingly confirmed by investigations in Hungary. Arany[1] and de 'Sigmond[28] have shown that an important type of alkali soil of Hungary has been markedly ameliorated by the application of sugar beet lime. Before the sugar beet lime was applied the Hungarian soil did not contain $CaCO_3$. It was in fact somewhat acid. Nevertheless, the soil contained relatively much replaceable sodium. It appears that this soil has been subjected to conditions in the state of nature which leached away the soluble products of the hydrolysis of its Na-clay, with the result that the soil mass has become slightly acidic owing to the accumulation of H-clay. Hence the soil contains both Na-clay and H-clay. It is probable that upon applying lime a reaction was set up with the H-clay of the soil which brought about the formation of Ca-clay and $Ca(HCO_3)_2$. The calcium thus made soluble replaced sodium from the exchange complex with the consequent conversion of Na-clay into Ca-clay. Thus the application of lime brought about a diminution in replaceable sodium and an increase in replaceable calcium. These chemical transformations produced marked flocculation of the soil, and hence an improvement in its physical properties and in the growth of crops.

It is important to state in this connection that the beneficial effect of lime as a treatment for alkali soil is probably limited to acid (the so-called degraded) types of alkali soil, for it is obviously unreasonable to expect any important effect from liming, if the soil already contains $CaCO_3$. In unpublished investigations at Fresno, for example, E. E.

Thomas has obtained no benefit whatever from the application of $CaCO_3$, either in the form of ground limestone or as sugar beet lime, but as stated above, this soil contains considerable $CaCO_3$. It may also be mentioned in passing that $CaCO_3$ is widely distributed in the semiarid regions of America. Generally speaking, the alkali soils of the American continent contain much more $CaCO_3$ than the Fresno soil. It is not probable, therefore, that lime will be useful in their reclamation.

From the foregoing discussion it is evident that any agency which will bring about an increase in the solubility of the calcium minerals of the soil will promote the reclamation of a sodium saline soil. This is true because the rate of replacement of sodium by calcium is roughly proportional to the concentration of the Ca ions in the soil solution. It is largely for this reason that decaying organic matter and the generation of CO_2 in the soil by microörganisms and by plant roots exert a favorable influence on the reclamation process. Consequently the growth of alkali-resistant crops as green manures may be utilized as a practical means of promoting the reclamation process.

The above interpretation of the role played by $CaCO_3$ in alkali soil reclamation is in agreement with the views of Gedroiz as set forth in detail in his publication of 1928.[11] However, he again emphasized the view that when subjected to leaching an alkali soil will remain toxic to plants as long as it contains important amounts of replacement sodium, owing to the fact that a toxic concentration of Na_2CO_3 will be continually present. Moreover, he pointed out that, since $CaCO_3$ promotes the formation of Na_2CO_3 through replacement of sodium by calcium from the base-exchange complex, the soil might even become less favorable for crop growth when leached than if $CaCO_3$ were absent. The authors' experimental results do not support this conclusion.

From the preceding discussion it follows that the development of alkalinity as a result of leaching a sodium saline soil, should not be interpreted to mean that the soil has necessarily been injured by the leaching process. If the soil contains $CaCO_3$, the formation of Na_2CO_3 as a result of leaching denotes that sodium has been replaced by calcium, and therefore the leaching process has changed the base-exchange constituents in the direction of a normal soil. It is, of course, essential to remove the Na_2CO_3 thus formed, otherwise toxic concentrations of OH ions will ensue, and unless this be done the replacing action of $CaCO_3$ will be brought to a standstill owing to the low solubility of $CaCO_3$ and the high concentration of soluble sodium that will ensue.

The principal objection to the leaching process as a practical method of reclaiming sodium saline soil lies in two facts: (1) The soil may become so highly deflocculated, owing to the removal of soluble electro-

lytes and hydrolysis, as seriously to retard the penetration of water; (2) the rate of the reactions are relatively slow. The dialysis experiments referred to above have indicated that, were it possible to remove Na_2CO_3 from the soil speedily, the rate of the reaction between $CaCO_3$ and Na-clay would be very rapid, but unfortunately sodium-saturated soils become excessively dispersed upon leaching. The result is that the removal of Na_2CO_3 may become impossible unless some flocculating substance is applied.

The leaching method also has been objected to on the ground that the high dispersion of the clay constituents of the soil will cause the mechanical transfer, through elutriation, of the base-exchange constituents from the surface soil into the subsoil, and thus will impoverish the upper part of the soil profile of these important components.[6, 9] This conclusion, based on laboratory leaching experiments, has not been confirmed by the authors' studies on the Fresno soil. It has been found, for example, that the base-exchange capacity of this soil has not been diminished by leaching (table 7).

Gedroiz[10, 11] and de 'Sigmond[26, 27] hold that when sodium saline soil is subjected to prolonged leaching, there is developed first the solonetz structure, and later the solodi, or degredation condition, the latter denoting that more or less of the base-exchange complex has been decomposed. However, if the soil contains $CaCO_3$, Gedroiz[11] concluded that leaching will not produce decomposition of the inorganic constituents of the base-exchange complex except to a very limited extent. The authors' results indicate that the base-exchange complex of soil containing $CaCO_3$ undergoes no important decomposition upon leaching. This must be true, since H-clay, formed by hydrolysis from Na-clay, will immediately react with $CaCO_3$ and thus be converted into stable Ca-clay. It is a fact, however, that Na-clay becomes excessively deflocculated upon leaching and this is a serious objection to the leaching method. With heavy clay types of sodium saline soil, it is not probable that reclamation can be effected simply by leaching with water. It is also not probable that the leaching method, when unaccompanied by other treatment, will be successful with alkali soils having the physical structure of a solonetz, owing to the extremely impervious character of the subsoil.

Various theories have been proposed to explain the deflocculation of Na-clays.[30] The most generally accepted view is that OH ions, formed by hydrolysis, cause dispersion of the soil particles. However, the behavior of soil colloids saturated with different bases, as revealed by studies with the use of the petrographic microscope, suggests that some additional factor is involved. This subject will be more fully discussed in a separate paper.

EFFECT OF LEACHING ON THE pH OF THE SOIL

Finally, it is of interest to note that the pH of the Fresno soil has not been increased, but rather it has been definitely decreased by leaching (table 7). Soil samples, drawn from the check plot immediately after the termination of the leaching periods of 1920 and 1921, showed that leaching had materially reduced the pH of the upper part of the soil profile. Samples drawn at later dates showed still further reduction in the pH values. This was probably due both to the leaching out of the native Na_2CO_3 of the soil and to the replacement of sodium by calcium. The authors do not believe that leaching will cause an increase in the OH-ion concentration of soil which contains considerable Na_2CO_3.

WHITE-ALKALI SOILS

The alkali soil of the Imperial Valley, California, differs from the Fresno soil, discussed in the preceding section of this paper, in three important particulars: (1) It is free from soluble CO_3; (2) it is a Ca-Na saline containing a high concentration of soluble calcium; (3) the soil is a heavy clay loam. The soil and subsoil are fine-textured down to a depth of about 8 feet, below which a porous, sandy substratum is found. There are no well-defined structural horizons within the profile. It is therefore a Na-Ca solonchak.

In 1929 a series of plots were prepared for irrigation on a barren area of this soil near El Centro. Certain of these plots were treated with gypsum, others with sulfur, and still others with gypsum or sulfur and barnyard manure. Three plots were left untreated as checks. All of the plots were flooded with water from the Colorado River, analysis of which is reported in table 8, for three successive periods between December, 1929, and May, 1930. Alfalfa was sown in October, 1930. In 1931 and 1932 good yields of alfalfa were obtained from all the plots (figs. 4 and 5).

It was found that leaching without other treatment produced as good results as the application of gypsum or sulfur. The data reported in table 10 show that the leaching has removed the chief part of the soluble salts to a depth of at least 48 inches. Table 11 shows that this soil does not contain important amounts of replaceable sodium. Although the content of soluble sodium salts was originally very high, the concentration of soluble calcium and magnesium was sufficient to prevent base replacement by sodium. These results confirm the conclusions which Gedroiz[8, 11] and de 'Sigmond[25] drew chiefly on the basis of theoretical reasoning.
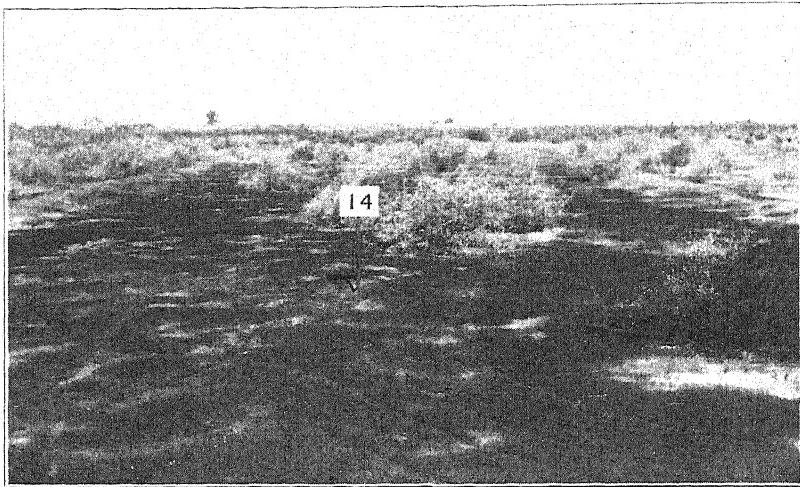
Fig. 4. This photograph, taken June 17, 1929, illustrates the general condition of the Imperial Valley soil before the reclamation experiment was begun.
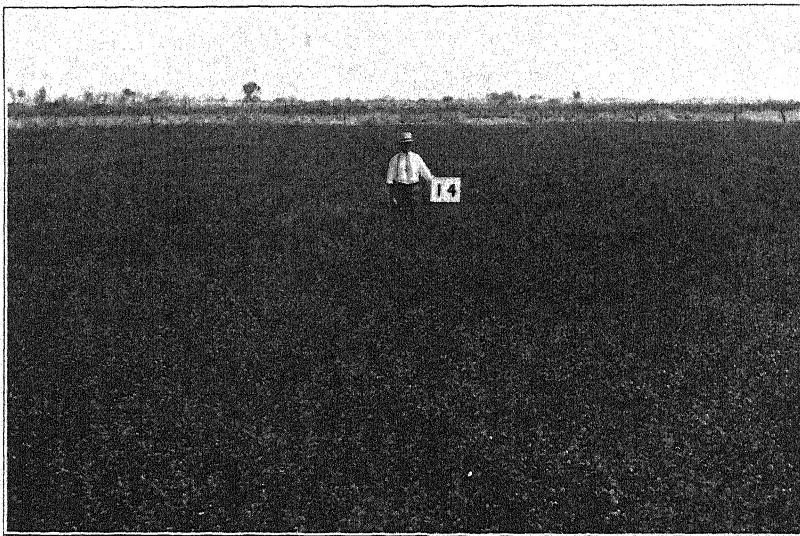

Fig. 5. This photograph (June 7, 1932), taken from the same place as that of figure 4, shows the growth of alfalfa after leaching the Imperial valley soil. In 1930 and 1931 good growth of alfalfa was also obtained on this plot.

This soil also contains about 10 per cent CaCO₃, but this constituent has played no important part in the reclamation process because of the abundance of soluble calcium salts which it contained. For the same reason it seems logical to assume that the calcium content of the irrigation water has played but little part in the experimental results. The reclamation of this type of alkali soil is, therefore, merely a matter of leaching out the excess of soluble salts.

TABLE 10

WATER-SOLUBLE SALTS, IMPERIAL VALLEY SOIL

(Milliequivalents per 100 grams)

Depth in inches	CO₃	HCO₃	Cl	SO₄	Ca	Mg	K	Na
Before leaching								
0–12	0.0	0.3	80.2	11.7	26.0	14.0	2.4	50.8
12–24	0.0	0.4	20.8	4.1	4.7	4.3	1.6	14.7
24–36	0.0	0.3	11.7	3.2	2.8	2.1	1.3	9.0
36–48	0.0	0.4	8.4	3.4	1.8	1.9	1.0	7.5
After leaching								
0–12	0.0	0.5	0.5	1.6	0.7	0.5	0.6	0.8
12–24	0.0	0.5	0.7	2.4	0.5	0.4	0.6	2.1
24–36	0.1	0.6	0.6	2.0	0.2	0.7	0.5	1.9
36–48	0.1	0.7	0.9	3.5	0.2	0.5	0.5	4.0

TABLE 11

REPLACEABLE BASES, IMPERIAL VALLEY SOIL

Depth in inches	Milliequivalents per 100 grams			Na as per cent of total
	Ca + Mg	K	Na	
Before leaching				
0–12	13.70	0.00	0.00	0
12–24	14.55	0.00	0.20	1
24–36	11.30	0.04	1.80	13
36–48	17.92	0.11	1.70	9
After leaching				
0–12	17.36	0.19	0.70	4
12–24	15.51	0.24	1.25	7
24–36	13.56	0.29	2.40	14
36–48	17.29	0.63	4.65	20

BASE-EXCHANGE CONSTITUENTS OF ALKALI SOILS

It seems desirable to present a brief discussion in this connection on the base-exchange constituents of alkali soils and of methods for the determination of the replaceable bases. Alkali soils commonly differ from normal soils not alone in containing an excess of soluble salts and replaceable sodium, but also in that the former may contain abnormal amounts of precipitated silicates and carbonates. It is well established that the base-exchange property of normal soils is due chiefly to the clays, which are alumino-silicates, and to the humates. It is believed that the importance of base exchange in alkali soils is also largely controlled by the clay and humus constituents, but the replaceable (absorbed) bases that are held by the clay and humus of alkali soils can be determined only by the use of special methods.

As Hilgard showed, the soluble salts of alkali soils are derived from the soluble products formed by the weathering of the rock masses within a given watershed. These soluble products become concentrated in certain places owing to the periodic nature and quantity of the precipitation. The soluble products of weathering are chiefly chlorides, sulfates, carbonates, bicarbonates, and small amounts of silicates. Soluble carbonates and bicarbonates may also be formed in the soil as a result of the hydrolysis of Na-clays and Na-humates.

As is well known, Na_2CO_3 is a solvent for SiO_2. The result is that more or less soluble silicate is likely to be formed within the soil mass wherever high concentrations of Na_2CO_3 accumulate. Alkali soils usually contain considerable $CaCO_3$. This substance, being soluble to some extent in solutions of NaCl and Na_2SO_4, gives rise to more or less dissolved Ca ions. Ca ions and Mg ions are also brought into solution by base exchange. Moreover, the soluble products of weathering may also include considerable calcium and magnesium. Therefore, the conditions that obtain in black alkali soils inevitably lead to the formation and precipitation within the soil mass of more or less calcium and magnesium as silicates and carbonates. Silicates and carbonates of the divalent bases may also accumulate in the soil to some extent in the absence of Na_2CO_3, since, upon concentrating the soluble products of weathering, the solubility of calcium and magnesium silicates and carbonates may be exceeded. It follows, then, that alkali soils of various kinds are likely to contain more or less precipitated silicates and carbonates. These facts must be taken into consideration if one is to arrive at an intelligent understanding of alkali soils.

With normal soils the replaceable bases are usually determined by extraction with a salt solution, or a dilute acid, or by electrodialysis. All these methods bring into solution more or less base from relatively simple silicates and carbonates of calcium and magnesium as well as from the clay and humus constituents. If, for example, precipitated calcium silicate or magnesium silicate be treated with a solution of $BaCl_2$, true base exchange ensues in which a part of the calcium and magnesium is replaced by Ba ions with the formation of $CaCl_2$ or $MgCl_2$ and insoluble barium silicate. However, if an ammonium or sodium salt be used instead of $BaCl_2$, calcium and magnesium are also brought into solution, but NH_4 ions are not absorbed to an important extent by these silicates, owing to the fact that ammonium silicate is unstable, and sodium silicate is, of course, soluble in water. Therefore, calcium silicate or magnesium silicate will not be converted into sodium or ammonium absorption compounds by reaction with soluble sodium or ammonium salts. On the other hand, the NH_4-clays and NH_4-humates and the corresponding sodium compounds are relatively stable and insoluble. The authors have taken advantage of these facts as a means of distinguishing between base exchange due to the clay and humus constituents, on the one hand, and reactions set up by the silicates and carbonates, on the other hand.

It is also well established that the absorption of cations by colloids is some function of the OH-ion concentration. Soils absorb greater amounts of cations from alkaline than from neutral solutions. In view of this fact, Bradfield[2] and Mattson and Hester[20] have suggested that base-exchange capacity should be defined in terms of pH. The term base-exchange capacity is used by the authors to denote the maximum amount of NH_4 the soil is able to absorb upon prolonged leaching with neutral normal ammonium acetate. The determination is made as follows: A sample of soil is leached with a solution of ammonium acetate as long as base is extracted from the soil. The uncombined ammonium acetate is then leached out with methyl alcohol, which practically prevents hydrolysis, after which the absorbed NH_4 is determined by aeration. The details of the method were described by Chapman and Kelley.[4]

Sodium and potassium are determined in the ammonium acetate extract of the soil, but calcium and magnesium are disregarded owing to the impossibility of measuring the extent to which precipitated silicates and carbonates have contributed to the base content of the extract. The content of water-soluble sodium and potassium must of course be subtracted from the results. This correction is not highly accurate owing to hydrolysis. Moreover, since leaching a black-alkali soil with neutral ammonium acetate changes its pH toward neutrality, its con-

tent of absorbed cations (NH_4), after having been leached with neutral ammonium acetate, will probably be less than the equivalent of the total bases that were removed from the soil. However, this method affords a means of determining the total content of absorbed sodium.

Gedroiz[9] suggested that replaceable (absorbed) calcium can be determined in alkaline soils by subtracting the calcium equivalent of the dissolved carbonate from the calcium found in the extract. This correction obviously rests on the assumption that $CaCO_3$ is the only carbonate present. A similar assumption underlies the titration method of Tjurin.[29] This assumption is not always justified, since $MgCO_3$ is sometimes present in soils. Moreover, black-alkali soils may also contain basic carbonate of magnesium.

In view of the above discussion, replaceable calcium and magnesium have not been determined individually, but, instead, the sum of the replaceable potassium and sodium was subtracted from the base-exchange capacity as determined by the absorbed NH_4. The result is reported as calcium and magnesium collectively.

DISCUSSION

There seems to be good reason for the belief that either one or the other of the above-named methods of reclamation, that is the application of a soluble calcium salt, or of some substance which will increase the solubility of the calcium minerals of the soil, or leaching without applying any soil amendment, is applicable to alkali soils everywhere, with the possible exception of the so-called degraded types of alkali soils. In connection with these investigations soil samples have been studied from many alkali areas of California and other states. In certain localities both farmers and scientific workers have been able to secure complete reclamation of important areas of alkali soil by applying the principles of one or the other of the above-named methods. Many of these soils contain considerable water-soluble calcium salts, and wherever this is the case leaching has proved to be a successful method of reclamation.

Black-alkali soils may or may not require the application of some substance, such as gypsum or sulfur. As shown already, the Fresno type of black-alkali soil can be reclaimed by mere leaching with water; but this soil responds to the leaching process too slowly to justify placing complete reliance on leaching as a practical method of reclamation. Certain other types of black-alkali soil, however (for example east and north of Great Salt Lake, Utah), respond to leaching readily. In this case the effectiveness of leaching is probably due to a combination of two fortunate circumstances, namely: (1) The composition of the irrigation

water which is relatively rich in calcium and magnesium salts and (2) the occurrence of precipitated $CaCO_3$ and calcium silicate, which are thoroughly disseminated throughout the profile of this soil.

The difficulty that must be overcome in the reclamation of alkali soils in general lies in their content of soluble salts and replaceable sodium, but alkali soils are usually not deficient in total calcium. Rather, replaceable forms of calcium may have been converted into other forms through base exchange and precipitation. Both $CaCO_3$ and calcium silicates may be caused to play an important role in the reclamation process. Any agency which will increase the solubility of these calcium minerals of the soil will promote the replacement of absorbed sodium by calcium and thus convert Na-clays and Na-humates into normal Ca-clays and Ca-humates. Among such agencies are elemental sulfur, CO_2 formed by plant roots and the decomposition of organic manures, iron sulfate, and soluble aluminum salts.[16] Obviously the absorbed sodium can also be replaced by applying a soluble calcium salt either as a constituent of the irrigation water or as a soil amendment.

From the foregoing it follows that the important properties of alkali soils are dependent on not only the soluble salts, as was emphasized by Hilgard, and the replaceable sodium, as was emphasized by Gedroiz, but also on the calcium minerals of the soil other than the base-exchange constituents. Furthermore, $CaCO_3$ of the soil and the calcium and magnesium salts of the irrigation water may play important parts in the reclamation process.

Other conditions being equal the facility of reclamation will be inversely proportional to the clay content of the soil. Heavy types of alkali soil may prove to be extremely difficult to reclaim, especially if the clay is largely saturated with sodium. Solonetz or solonetz-like subsoil horizons, which are high in absorbed sodium, will probably be exceedingly difficult to ameliorate. With alkali soils of this type the application of a calcium salt of high solubility should, on theoretical grounds, produce the best results.

The removal of the soluble salts and the complete replacement of sodium by calcium from the base-exchange complex of the soil are dependent on the possibility of leaching the soil, and it is obviously impossible to effect leaching if the ground water level remains near the surface, or if the soil is impenetrable to water. The drainage conditions are therefore exceedingly important in alkali soil reclamation. However, a general discussion of drainage would be inappropriate in this paper, since the drainage conditions do not alter the essential nature of the chemical principles which govern the reclamation process. For a similar reason a discussion of methods of practical management and of the sys-

tems of cropping and irrigation that will give the best results with a given alkali soil are also omitted from this publication, although the authors fully recognize that practical success of reclamation may largely depend on the observance of special care with respect to methods of irrigation and soil management.

SUMMARY

The history of present views as to the essential nature of alkali soils has been briefly reviewed. It was shown that alkali soils contain either an excess of soluble salts or abnormal amounts of replaceable (absorbed) sodium. The clay and humus of normal soils are combined with calcium. With alkali soils, on the other hand, soluble sodium salts tend to bring about replacement of calcium by sodium and thus produce chemical and physical conditions that are extremely adverse to plant growth. Hence, successful reclamation of alkali soils depends on the removal of the soluble salts and the replacement of the absorbed sodium by calcium.

The Russian word solonetz is used by soil investigators in two senses, namely, chemically and physically. In order to avoid confusion it is suggested that this term be used to denote both chemical and physical properties of the soil. Accordingly solonetz is defined as alkali soil containing replaceable (absorbed) sodium the profile of which presents certain morphological structures.

This paper reports the results of investigations on the reclamation of two important types of alkali soil, namely, the black-alkali soil of the Fresno area of California and the white-alkali soil, Imperial Valley, California. It has been shown that the application of gypsum or sulfur has produced markedly effective results on the Fresno black-alkali soil, while leaching without other treatment has been equally effective with the Imperial Valley white-alkali soil. The Fresno soil can also be reclaimed by simple leaching, but only at excessively slow rates. The Fresno soil contains much replaceable sodium (60 per cent or more of the total replaceable bases), while the white-alkali soil of the Imperial Valley of California, contains almost no replaceable sodium.

The important aspects of the chemistry of alkali soil as affected by treatments with gypsum and sulfur and by leaching are discussed in detail.

The composition of the irrigation water is an important consideration in connection with the reclamation of alkali soils.

The accomplishment of successful reclamation depends on the maintenance of effective drainage conditions.

The determination of replaceable calcium and magnesium in alkali

soil is especially difficult. A method is outlined for the determination of the replaceable divalent bases collectively.

The following points should be carefully considered before proceeding to reclaim an area of alkali soil: (1) The drainage conditions; (2) the composition of the soluble salts; (3) the content of replaceable (absorbed) sodium in the soil; (4) the nature and content of the calcium minerals of the soil; (5) the composition of the available irrigation water.

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DENSITY AND ARRANGEMENT OF VINES[1]

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THE DENSITY OF VINES in the vineyard, or ratio of population to unit area, varies greatly in the various grape-growing regions of the world. The arrangement, or relation of position of the individual vines to each other, varies in a similar way. The density ranges from about 10,000 to the acre in Champagne to about 200 in Almeria. The arrangement varies from *promiscuous* (irregular) to various regular systems, of which the most common are forms of the *rectangular* (square and avenue). The hexagonal system, formerly common, is little used now in California.

Density.—What is the most suitable population depends upon many conditions—soil, climate, water supply, character of available labor, and the capacity for growth of the variety of vine. Conditions which restrict the growth of the vine—ecological factors or the intrinsic nature of the vine—usually require the greater densities to insure full acre yield. In Champagne and regions of similar cool climate close planting is believed to promote shallow rooting and thereby to be favorable to the early ripening of the grape. On the other hand, the more favorable the climate, the more fertile the soil, and the greater the capacity of the variety for growth, the less the density should be. Where these favorable conditions exist, close planting results in a crowding and interlacing of the canes and a dense shade of foliage which interferes with the setting, growth, coloring, and ripening of the fruit, increases the difficulty of control of fungus and insect enemies of the vine, and makes cultivation and harvesting unduly costly.

The water supply—from rain or irrigation—is also a limiting factor. If it is deficient, a small number of vines may succeed where a larger number competing for the inadequate supply would fail to yield profit-

able crops. Where water is the limiting factor, it may be exhausted by a dense population before the crop has matured.

Arrangement.—The most advisable arrangement of the vines is determined by the methods of cultivation used, the system of training and harvesting adopted, and by what appears to give the most perfect utilization of the soil. Where most or all of the cultivation is done by hand, as in Champagne, the promiscuous arrangement is satisfactory. For densities of 5,000 or more this is the only practical method, but for densities of less than 3,000 most of the cultivation can be facilitated by the use of draft animals and in this case a regular arrangement of the vines is necessary. Where mechanical tractors are used, the density with the square system cannot conveniently be more than 700, while for greater economy and with large implements it must be reduced to 500 or even 300. With the avenue system where the vines are trellised and cultivated in only one direction somewhat greater densities may be used.

Density and Arrangement.—What is the best combination of density and arrangement for any particular set of circumstances can be determined only by long experience or by carefully conducted experiments. When found, it will be a compromise decided by the quantity and quality of the resulting crops and the cost of the various cultural and harvesting operations. The costs of production per acre and per ton will generally decrease with decrease of density up to an optimum for the particular conditions, owing to the saving of hand labor by the use of machines, and at the same time the quality of the crop may be improved by the more favorable aeration and insolation of the fruit. The quantity of the crop will, on the other hand, increase with the increase of density up to a certain point, which varies with the other factors discussed. The quantity will be greatest with the arrangement which enables the vines to utilize the soil area most effectively.

Of the various arrangements, it is generally considered that the hexagonal system is most favorable to the early and complete utilization of the soil and the wide avenue system the most unfavorable. This consideration is based on the assumption that a young vine spreads its roots equally to all points of the compass. If this assumption is correct, the periphery of the root system will be a circle. This circle will enlarge until it meets the corresponding circles of the adjoining vines. This, it is further assumed, brings about competition, and further extension is stopped or retarded. When this theoretical point is reached with the hexagonal system, the root circles of the vines occupy 91 per cent of the soil area. With the square system, they will occupy only 79 per cent, and with the avenue system they will occupy a percentage increasingly less as the difference between the width of the avenues and the distance
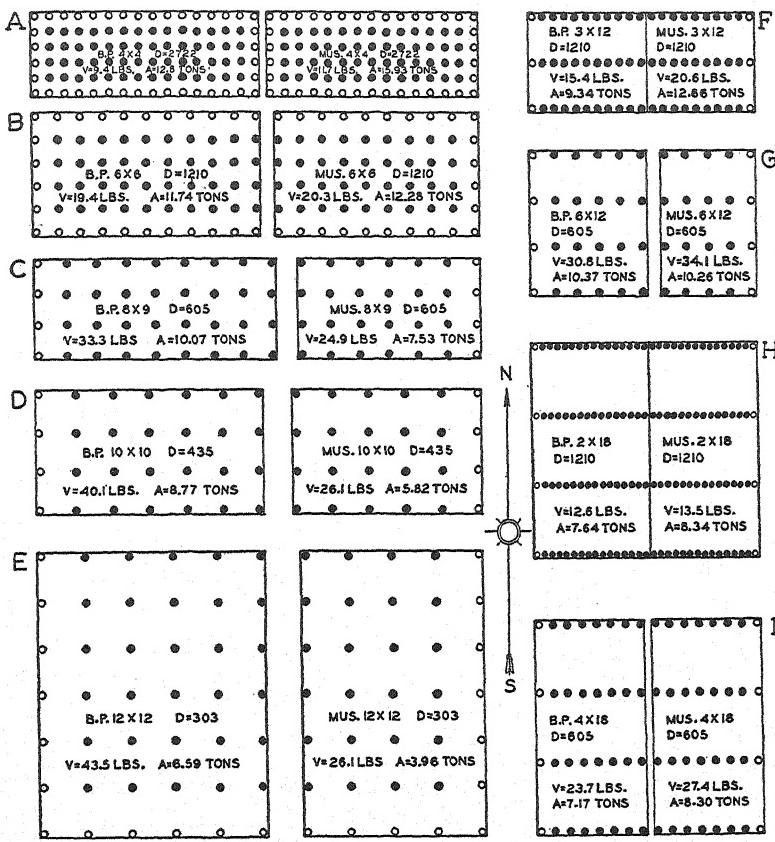
Fig. 1. Plan of the density and arrangement vineyard. The yields given are means of the crops of 1926, 1927, and 1928.

TABLE 1

HYPOTHETICAL UTILIZATION OF SOIL WITH VARIOUS
ARRANGEMENTS OF VINES

Arrangement and distance in feet	Hypothetical utilization of soil at the beginning of competition	
	Formula used*	Per cent
Hexagonal	$O \div T = \pi \div 2\sqrt{3}$	90.69
Square	$O \div T = \pi \div 4$	78.54
Avenue	$O \div T = \pi r \div 2(2r+a)$	....
8×9	$O \div T = \pi r \div 2(2r+a)$	69.80
6×12	$O \div T = \pi r \div 2(2r+a)$	39.30
3×12	$O \div T = \pi r \div 2(2r+a)$	19.60
4×18	$O \div T = \pi r \div 2(2r+a)$	17.50
2×18	$O \div T = \pi r \div 2(2r+a)$	8.70

* $r = \frac{1}{2}$ vine distance; a = row distance − $2r$; O = area of root circle; T = total area per vine.

between the vines in the row increases. (See table 1 and fig. 1.) Whether the small difference between the hexagonal and square system is of any importance is doubtful, but there is very probably a retardation of development and a loss of crop, at least for some years, with the avenue system.

EXPERIMENTAL METHOD

Numerous accounts of experiments to determine the relation of density and arrangement to the quantity and quality of the vintage have appeared in viticultural literature during the past forty years, but the results have in most cases been doubtful owing to a failure to eliminate or equalize complicating conditions of soil, variety, or injurious effects of pests, diseases, or unfavorable weather.

Plan of the Experiment.—In order to obtain evidence on this problem, under normal or usual conditions of grape growing in California, an experiment was started at Davis in 1923 and continued until 1930, when it was abandoned on account of the invasion of the 4×4 Black Prince plots by phylloxera. All the 4×4 vines including the Muscat were removed in 1928. Fortunately, four crops, those of 1925, 1926, 1927, and 1928, were obtained except from blocks A and B in 1928. During the last three of these crop years it is probable that the hypothetical competition had begun among the more closely planted vines. The data obtained therefore should throw light on some of the effects of the various densities and arrangements tested.

The plan of the experiment was to limit the inquiry to the effects of variations of density and of arrangement on a variety with great capacity for growth and on a variety with moderate capacity, all other conditions being as uniform and favorable as possible.

The strong-growing variety chosen was the Black Prince and the weaker the Muscat of Alexandria. The soil of the experiment plot is fertile, deep, well supplied with water, and very uniform. The climatic conditions are favorable.

The details of planting are shown in figure 1.

The densities and arrangements chosen represent the commoner practices in use in California (plots C, D, and G), and extensions of these practices in the direction of greater density (plots A, B, F, and H), and of less density (plot E). They also include variations in the arrangement of the avenue plantings in the direction of widening the avenue with the same density—i.e., reducing the distance between the vines in the row and increasing correspondingly the distance between rows

(plots G, I, F, and H). The intention is to pass in each direction beyond the probable optimum of density and arrangement in order to be sure of including the optimum.

The treatment of all plots was as nearly as possible identical in the matters of cultivation, pruning, and other operations, except that the vines in the avenue system were trellised and cordon-pruned and that irrigation was applied to each plot according to need.

Data Collected.—The data collected were the crop, the circumference of the trunk, the weight of the prunings for each vine and each year, and finally the weight of each vine when removed at the conclusion of the experiment. From this material it is possible to obtain directly or to compute approximately the rate of growth and total size of the vine and the rate of increase and total weight of the crop.

With these results the influence of the variations in density, arrangement, and variety of vine can be estimated as illustrated by figures 2, 3, and 4 and the corresponding tables.

GROWTH OF THE VINES

Figure 2 shows the variations in rapidity of development and in total growth during the eight years of the experiment, resulting from variations in density, arrangement, and variety of vine.

Variety.—The Black Prince exceeded the Muscat in all parallel cases. The mean total growth of all the Muscat vines in the plots planted in squares was only 47 per cent of that of the Black Prince planted in the same way and only 40 per cent in the plots planted in the avenue system. This indicates a relative capacity for growth under the conditions of the experiment of 43.5 for the Muscat of Alexandria as compared to 100 for the Black Prince.

Arrangement.—An estimate of the influence of arrangement can be made by comparing plots C, G, and I, each with a density of 605 vines to the acre but with planting arrangements of 8×9, 6×12, and 4×18 respectively; and of plots B, F, and H, each with a density of 1,210 vines to the acre but arranged 6×6, 3×12, and 2×18 respectively.

Table 3 shows diminished growth with each increase in the difference in the distance between rows and in the distance between vines in the row. This restriction of growth is greater with the Muscat than with the Black Prince and is fairly regular in each case. It is an average of 29 per cent for the Black Prince and 45 per cent for the Muscat. This is much less than the hypothetical loss, which averages 71 per cent, and indicates that the theoretical loss due to competition is exaggerated or

TABLE 2

GROWTH OF VINES, 1923–1930

		Square System					

Plot	Distance in feet	Weight in pounds					
		1923	1924	1925	1926	1927	1930

		Black Prince					
A	4×4	0.73	2.05	4.05	5.83	6.66	*9.90**
B	6×6	0.81	2.86	6.20	9.24	11.02	*17.29*
C	8×9	0.86	3.48	8.34	13.33	16.91	*24.42*
D	10×10	0.90	4.27	9.83	15.84	20.09	*28.93*
E	12×12	0.86	4.69	10.52	17.38	22.22	32.23

		Muscat					
A	4×4	0.37	1.34	2.84	3.67	4.49	*5.70*
B	6×6	0.57	2.05	4.60	6.58	7.90	*9.88*
C	8×9	0.59	2.44	5.90	9.37	11.73	*11.88*
D	10×10	0.66	2.66	5.96	9.59	12.25	*12.76*
E	12×12	0.51	2.55	5.92	9.72	12.30	12.76

		Avenue System					

Plot	Distance in feet	Weight in pounds					
		1923	1924	1925	1926	1927	1930

		Black Prince					
F	3×12	0.81	2.38	5.10	6.64	8.14	12.98
H	2×18	0.46	2.05	4.31	5.98	7.22	9.13
G	6×12	0.95	2.79	6.29	8.78	11.09	20.24
I	4×18	0.84	2.60	5.90	8.47	10.76	17.82

		Muscat					
F	3×12	0.48	1.36	2.97	3.70	4.62	5.5
H	2×18	0.44	1.23	2.51	3.21	3.92	4.4
G	6×12	0.55	1.85	4.00	5.08	6.16	7.3
I	4×18	0.55	1.61	3.61	4.99	5.94	6.8

*Italic figures are estimates based on the weighings of the other plots. Other figures in 1930 are actual weighings.

that competition has not reached its maximum at eight years. It is probable that each of these suppositions accounts for part of the difference.

Density.—The influence of the density of population is shown by plots A, B, C, D, and E all planted with the square arrangement.
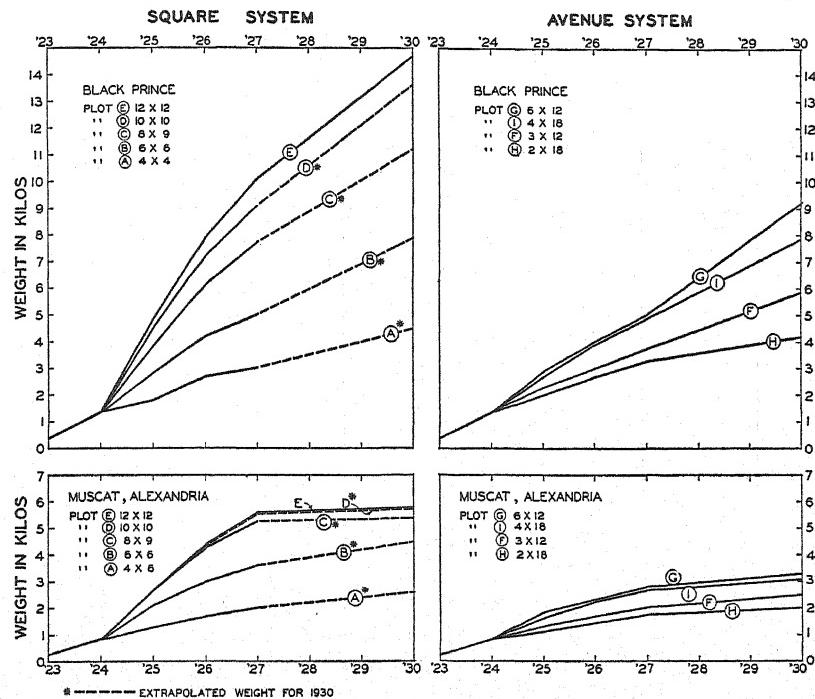

Fig. 2. Growth of vines, 1923–1930.

Table 4 shows an uninterrupted decrease in weight of vine with increase in number of vines per acre, with both varieties. The decrease is very regular with the Black Prince and follows a curve very close to that of decrease in distance between vines, indicating that competition between the vines had begun at least in the eighth year in the 10×10 plot and at increasingly earlier dates in the denser plots. With the .Muscat, competition does not appear to have begun in the eighth year at lower densities than 8×9 or 6×6, and the decrease of weight of vine is much less than with the Black Prince. The slower growth of the Muscat has evidently delayed the start of competition and has perhaps obviated it in the wider spacings. The superior vigor and more rapid development of the Black Prince fit it better for the wider spaces. It has made 152

per cent more growth than the Muscat in the widest spacing but only 75 per cent more in the narrowest.

The assumption that the size of the vines will finally be proportional to the available space allotted to each vine is probably approximately true within limits when all other conditions are equal. If this assumption is true for the conditions and limits of this experiment, the point of com-

TABLE 3

INFLUENCE OF ARRANGEMENTS OF VINES ON GROWTH

Plot	Distance in feet	Density, in vines per acre	Average weight per vine at eight years				Vd*	$(Vd)^2$†
			Black Prince		Muscat			
			Pounds	Per cent of plot in square system	Pounds	Per cent of plot in square system		
C	8× 9‡	605	24.42	100	11.88	100	100	100
G	6×12	605	20.24	83	7.26	61	75	56
I	4×18	605	17.82	73	6.82	57	50	25
B	6× 6	1210	17.29	100	9.88	100	100	100
F	3×12	1210	12.98	75	5.50	56	50	25
H	2×18	1210	9.13	53	4.40	45	33	11

* Vd=The theoretical percentage of the plot in the square system if growth varied as the distance between vines in the row.

† $(Vd)^2$=The theoretical percentage of the plot in the square system if growth varied directly with the soil area per vine, i.e., with the square of the distance between vines in the row.

‡ The variation of 8×9 from the square is neglected.

plete utilization of the space for all plots had not yet been reached; for although the vines in the wider plantings are larger in virtually all cases, they have not yet reached the size represented by the available space.

CROP PER VINE AND CROP PER ACRE

If the mean crop per vine varied inversely as the number of vines per acre, the crop per acre would be constant. It seems probable that *within certain limits of density,* varying with the conditions of soil, climate, and variety, this will be true when the vines reach the stage of development where they are utilizing fully the available space. This stage will. be the more remote the wider the spacing; and if the spacing is so wide that its utilization is beyond the capacity of the variety, the maximum crop possible with closer spacing will not be attained. For densities which allow the vines to utilize all the available space, but which are not so dense as to crowd the vines and thus interfere with the supply of

light and air needed for growth and production, it is probable that the crops would finally vary little in volume. The increase of crop per vine for the two varieties and the nine plots during the four years of bearing is shown in figure 3.

Square System.—With the Black Prince, after the first crop, the wider spacings gave the larger yields *per vine* in all the square-planted plots. The difference was noticeable in the first commercial crop and continued during the life of the experiment. This indicates that competition between adjacent vines, which was evident in the third year

TABLE 4

INFLUENCE OF DENSITY OF PLANTING ON GROWTH OF VINE

Plot	Dinstance in feet	Density, in vines per acre	Average weight per vine at eight years				Vd*	$(Vd)^2$†
			Black Prince		Muscat			
			Pounds	Per cent of plot E	Pounds	Per cent of plot E		
E	12×12	303	32.25	100	12.76	100	100	100
D	10×10	435	28.93	89	12.76	100	83	70
C	8× 9	605	24.42	76	11 88	92	71	50
B	6× 6	1210	17.29	54	9.88	76	50	25
A	4× 4	2722	9.90	31	5.70	45	33	11

* Vd=The theoretical percentage of plot E if growth varied as the distance between vines.

† $(Vd)^2$ =The theoretical percentage of plot E if growth per vine varied directly with the soil area per vine.

(1925) by the growth of the vines (fig. 2) affected the crop notably the fourth year. With the Muscat a distinct increase of crop *per vine* occurred only up to the 8 × 9 plot, the crop of which was virtually identical with those of the 10 × 10 and 12 × 12 plots up to the fifth year (1927) and showed little difference in the sixth. This indicates that the relatively small-growing Muscat had nearly or quite reached its fullest development in the 8 × 9 plot, while the stronger-growing Black Prince was still profiting by the increased space up to at least the 10 × 10 plot. The crops of all square-planted plots were slightly smaller in the sixth year than in the fifth, owing to a less favorable season. The widest plantings of the Muscat as well as those of the Black Prince apparently suffered less than the closest, indicating perhaps that there existed some competition even in the widest planting, though this is hardly evident in the growth. The mean weight of the Muscat vines in 1930 (fig. 2) showed little increase in the wider plantings due to greater soil area per vine, being 5.4, 5.8, and 5.8 in plots 8 × 9, 10 × 10, and 12 × 12, respectively. The increases in weights of the Black Prince vines in corresponding plots were somewhat larger—11.1, 13.6, and 14.7; or in per-

centages, 100, 107, and 107 for Muscat, and 100, 123, and 132 for Black Prince.

This seems to indicate that for the conditions of soil, climate, and treatment of the experiment, the Muscat would give its maximum crop per vine with a density of about 600 vines to the acre arranged in squares, but that the Black Prince under the same conditions would still increase its mean crop per vine with a density of 300 or lower.
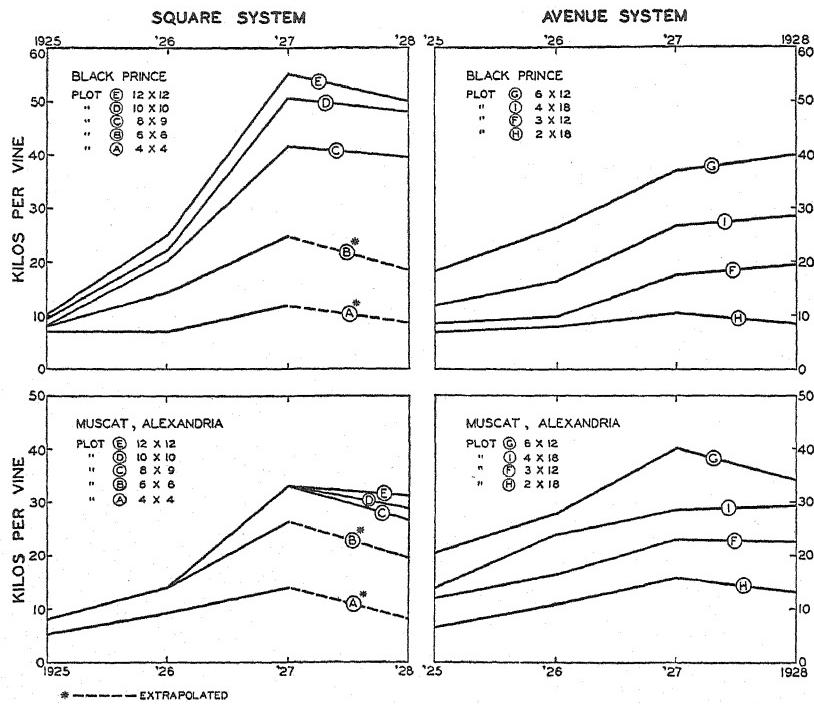

Fig. 3. Increase of crop per vine, 1925–1928.

The increased crop per vine was in no case sufficient to compensate entirely for the smaller number of vines. With 2,722 vines to the acre, the Black Prince yielded in the four crops 47.1 tons per acre though the mean total crop per vine for the four years was only 34.6 pounds, while with 303 vines per acre and a mean total crop per vine of 140.4 pounds, it yielded only 21.3 tons. This is an increased vine yield of over 300 per cent and a decreased acre yield of 55 per cent.

With the Muscat the corresponding figures are: with 2,722 vines, 50.6 tons per acre and 37.2 pounds per vine, and with 300 vines, 12.8 tons per acre and 84.9 pounds per vine, or a gain of 128 per cent in crop per vine and a loss of 75 per cent in crop per acre for the wider spacing.

This comparison undoubtedly exaggerates the loss of crop due to the lower vine concentrations because the more populous plots had more nearly reached their maximum crops than the less populous. A better comparison is that shown in table 6 where it can be seen that the loss of

<div align="center">

TABLE 5

INCREASE OF CROP PER VINE, 1925–1928

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Plot	Distance, in feet	Density, in vines per acre	Annual crop, in pounds per vine				Total crop	
			1925	1926	1927	1928	Pounds per vine	Tons per acre
Black Prince in square system								
E	12×12	303	10.0	25.0	55.2	50.2	140.4	21.3
D	10×10	435	9.4	22.6	50.1	47.7	129.8	28.3
C	8× 9	605	8.1	19.5	41.3	39.2	108.1	32.8
B	6× 6	1,210	9.2	14.1	24.7	18.5	66.5	40.2
A	4× 4	2,722	7.0	7.1	11.8	8.8	34.6	47.1
Black Prince in avenue system								
G	6×12	605	20.5	26.2	36.6	39.6	120.9	36.6
I	4×18	605	11.9	16.2	26.3	28.6	82.3	24.9
F	3×12	1,210	9.2	9.5	17.4	19.4	56.4	34.1
H	2×18	1,210	6.4	8.1	15.0	14.8	44.2	26.7
Muscat of Alexandria in square system								
E	12×12	303	6.6	14.1	32.8	31.4	84.9	12.8
D	10×10	435	7.9	15.4	33.7	29.1	86.1	18.7
C	8× 9	605	9.0	14.7	33.0	27.0	83.7	25.3
B	6× 6	1,210	8.8	14.1	26.4	19.7	69.1	41.8
A	4× 4	2,722	5.9	9.2	14.1	8.0	37.2	50.6
Muscat of Alexandria in avenue system								
G	6×12	605	21.0	27.5	39.9	34.2	122.6	37.1
I	4×18	605	13.9	24.3	28.6	29.4	96.2	29.1
F	3×12	1,210	12.1	16.3	22.9	22.5	73.8	45.1
H	2×18	1,210	7.7	11.5	15.4	13.6	48.2	29.2

crop from larger spacing is diminishing in all cases. It indicates that it might disappear entirely in a few years with the Black Prince in all plots.

With the Muscat, though the loss shows decrease, it appears unlikely that it would disappear entirely except in plot B and perhaps in plot C, under the conditions of this experiment.

Avenue System.—In the plots arranged according to the square system there are two variable factors—variety and concentration. In the plots arranged according to the avenue system there is an additional

TABLE 6

CROP IN POUNDS PER VINE AND TONS PER ACRE; MEAN CROP, 1927–1928

Plot	Density, in vines per acre	Black Prince				Muscat			
		Mean yield, 1927–1928		Per cent of crop in plot A		Mean yield, 1927–1928		Per cent of crop in plot A	
		Pounds per vine	Tons per acre	1927–1928	1925–1928	Pounds per vine	Tons per acre	1927–1928	1925–1928
E	303	52.7	8.0	57	45	32.1	4.9	32	25
D	435	48.9	10.6	76	60	31.4	6.8	45	37
C	605	40.3	12.2	87	70	31.0	9.4	62	50
B	1,210	21.6	13.1	94	85	23.1	14.0	93	82
A	2,722	10.3	14.0	100	100	11.1	15.1	100	100

TABLE 7

COMPARISON OF CROPS PER ACRE WITH DIFFERENT ARRANGEMENTS AND WITH HEAD AND CORDON PRUNING

Plot	Distance in feet	Density, in vines per acre	Black Prince				Muscat			
			Mean yield, 1927–1928		Yield per acre in per cent of square system*		Mean yield, 1927–1928		Yield per acre in per cent of square system*	
			Pounds per vine	Tons per acre	1927–1928	1925–1928	Pounds per vine	Tons per acre	1927–1928	1925–1928
C	8× 9*	605	40.3	12.2	100	100	30.0	9.1	100	100
G	6×12	605	38.1	11.5	94	112	37.0	11.2	123	147
I	4×18	605	27.5	8.3	68	76	29.0	8.8	97	115
B	6× 6	1,210	31.6	13.1	100	100	23.0	13.9	100	100
F	3×12	1,210	18.4	11.1	85	85	22.7	13.7	98	108
H	2×18	1,210	14.9	9.0	69	66	14.5	8.8	64	70

* Variation of 8 × 9 from the square system is neglected.

variable—the method of pruning. In the square-system plots, the vines were head pruned, in the avenue system they were cordon pruned. We have thus two variables of opposing character—(*a*) increasing nearness of the vines in the row which tends to diminish the crop, and (*b*) cordon pruning which tends to increase it.[4] Table 7 is arranged to show the effect of the three variables.

If we compare the yields of the last two crops of the Black Prince in plots G and I of the avenue system with the yields for the same crops in

[4] At least with young vines.

plot C of the square system—all with the same vine concentration of 605 vines to the acre—we find that plot G arranged 6 × 12 has produced a crop 6 per cent less than plot C arranged 8 × 9 or virtually of the same amplitude; while in plot I arranged 4 × 18 the crop is 32 per cent less. This indicates that the restriction of crop due to narrowing the vine distance from 8 to 6 is compensated for by the augmentation of the crop due to cordon pruning. In plot I, however, the narrowing of vine distance from 8 to 4 has reduced the crop 32 per cent even with the augmentation presumably caused by cordon pruning. Comparing in the same way avenue plots F and H cordon pruned with the square plot B head pruned, all of concentration 1,210 to the acre, we find the narrowing of vine distance from 6 to 3 has reduced the crop 15 per cent and a narrowing from 6 to 2, 31 per cent, notwithstanding the cordon pruning.

Comparing the Muscat plots in the same way we find that the Muscat has been less unfavorably affected by narrowing the vine distances. The restriction of crop by narrowing the vine distance from 8 to 6 combined with the augmentation due to cordon pruning has resulted in a net *augmentation* of crop of 23 per cent, while narrowing from 8 to 4 and from 6 to 3 have had virtually no net effect on the weight of crop. The net reduction of narrowing from 6 to 2, however, is 36 per cent.

These differences from the results with the Black Prince seem to indicate that the Muscat benefited more from cordon pruning than the Black Prince or that it failed to benefit as much as the stronger-growing Black Prince from the wider vine distances. Probably both factors are involved.

SUMMARY AND CONCLUSION

The object of this experiment was to determine the influence on the fruitfulness of vines of their density and arrangement in the vineyard under identical and favorable conditions of growth.

Density.—It was found that under the climatic and cultural conditions of the vineyard at Davis the yield per vine at the *first crop* (in 1925, the third year after planting) was little, if at all, affected by the density. At all planting distances tested, from 4 × 4 ft. to 12 × 12 ft., either with the very vigorous Black Prince or with the more moderate Muscat of Alexandria, the first yield per vine was virtually the same. The first crop per acre, therefore, varied almost exactly with the density (the number of vines per unit area). With each subsequent crop up to 1928 the yield per vine, and therefore per acre, increased at all densities, but more rapidly with the wider spacings than with the closer (table 8).

As shown in table 8, the advantage of the closer plantings diminished

with time. In the 1925 crop, the yield per acre of the 4×4 planting of Black Prince was 6.3 times that of the 12×12 planting; and that of the 4×4 planting of Muscat, 8.0 times that of the 12×12. These differences gradually diminished until in 1927–1928 the corresponding ratios were 1.8 for the Black Prince and 3.1 for the Muscat. The ratio of increase in acre crop (i.e., the ratio of the 1927–1928 crop to the 1925 crop) was progressively higher with the wider spacing up to and including the 10×10 with Black Prince and the 12×12 with Muscat.

TABLE 8

INCREASE OF YIELD PER ACRE IN THE FIRST FOUR CROPS AT VARIOUS DENSITIES
(Square system)

Variety and crop	4×4	6×6	8×9	10×10	12×12	Ratio, 12×12 to 4×4
Black Prince						
1925, in tons per acre..........	9.5	5.6	2.5	2.0	1.5	6.3
Mean, 1927–1928, tons per acre......................................	14.0	13.0	12.2	10.6	7.9	1.8
Ratio 1927–1928 to 1925........	1.47	2.32	4.87	5.32	5.28	....
Muscat						
1925, in tons per acre..........	8.0	5.3	2.7	1.7	1.0	8.0
Mean, 1927–1928, tons per acre......................................	15.1	14.0	9.1	6.9	4.9	3.1
Ratio 1927–1928 to 1925........	1.89	2.64	3.36	4.05	4.86	....

It seems probable, from the trends indicated, that the differences in acre crop would in time disappear with the Black Prince or even be reversed in sign at these densities. With the Muscat, the approach to equality of acre crop was slower than with the Black Prince and indicates that the yield of the widest plantings, 10×10 and 12×12, might never equal that of the greater densities or at least would require many years.

These results suggest that under the conditions of the Davis vineyard, densities of from 300 to 500 vines to the acre, with the square system of planting, are suitable for obtaining maximum crops with Black Prince and similar strong-growing varieties. For Muscat and other moderate growers, the production of the largest crops would require about 600 vines to the acre, and perhaps more for weaker-growing varieties. The gain in larger crops with greater densities than these is evanescent and, under the conditions of this experiment, which are typical of a large part of the grape-growing regions of California, would usually be more than neutralized finally by loss due to the extra cost of vineyard work of all kinds—pruning, staking, tying, cultivation, irrigation, distribu-

tion of manure and boxes, and the handling of the grapes during harvesting.

Arrangement.—The influence on the crop of different arrangements of the vines where the density of planting was the same is shown in table 9 and figure 4, Sections III and IV.

With a density of 605 the yield per vine of the *Black Prince* at the first crop was 20.5 pounds in the 6 × 12 plot and 11.9 pounds in the 4 × 18 plot, or a decrease of *42 per cent* due to the reduction of the vine

TABLE 9

CROP IN POUNDS PER VINE, WITH VARIOUS ARRANGEMENTS

Variety	Year	Density 605 vines per acre			Density 1,210 vines per acre		
		Square system 8×9	Avenue system		Square system 6×6	Avenue system	
			6×12	4×18		3×12	2×18
Black Prince {	1925	8.1	20.5	11.9	9.2	9.2	6.4
	1928	39.2	39.6	28.6	18.5	19.4	14.8
Muscat.......... {	1925	9.0	21.0	13.9	8.8	12.1	7.7
	1928	27.0	34.0	29.4	19.7	22.5	13.6

distance from 6 to 4, or 33 per cent. With the *Muscat* the corresponding figures are 21 pounds in the 6 × 12 plot and 13.9 pounds in the 4 × 18 plot, or a decrease of *34 per cent.*

With a density of 1,210 the yield per vine of the *Black Prince* was 9.2 pounds in the 3 × 12 plot and 6.4 pounds in the 2 × 18, a decrease of *30 per cent,* and with the *Muscat* the corresponding figures are 12.1 pounds for the 3 × 12 plot and 7.7 pounds for the 2 × 18 plot, a decrease of *36 per cent;* the reduction of the vine distance being from 3 to 2 or 33 per cent as with the 605 density.

With each variety therefore the decrease of yield per vine at the first crop was very close to the 33 per cent reduction in vine distance. With later crops the decrease of yield was less in the plots of density 605, being 28 per cent with the Black Prince and 14 per cent with the Muscat at the fourth crop. In the plots of density 1,210, the decrease of yield was only slightly less with the Black Prince, 24 per cent, and with the Muscat the decrease was greater than with the first crop, or 40 per cent. A reduction of vine distance from 6 to 4 appears to have decreased the later crops of the Black Prince more than those of the Muscat, but a reduction in distance from 3 to 2 had an opposite effect, decreasing the crop of Muscat more than that of the Black Prince. A possible explanation of this difference is that the strong-growing Black Prince was better able to make use of the soil in the wide avenues than the weaker Muscat.

The crops of the 8 × 9 and 6 × 6 plots given in table 9 are not directly comparable with the avenue plots because of the different system of pruning. The use of long canes in forming the cordons in the third year accounts for the heavier crops in the avenue plots. The spur pruning used the same year in forming the head restricted the crop of the square-planted plots. By the fourth crop, however, the yields were not very
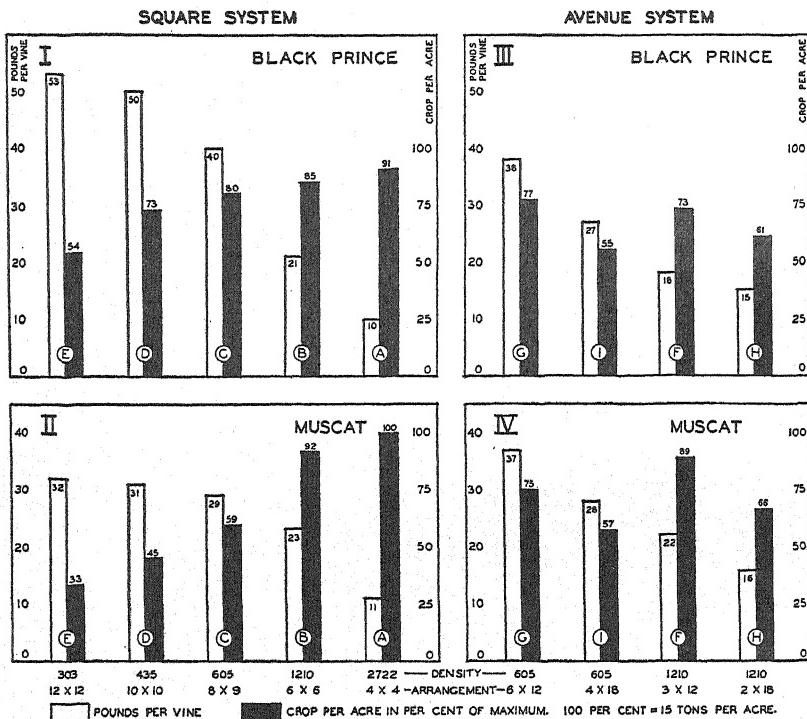
Fig. 4. Mean crop per acre and crop per vine, 1927–1928. Sections I and II show the square-system plots of the two varieties arranged in the order of increasing density. As the density increased, the crop per acre increased and the crop per vine decreased. In no case was the increase in vine crop sufficient to compensate entirely for the decrease in the number of vines.

Sections III and IV show the avenue-system plots of the two varieties. With each variety there were two sets of two plots each which were of equal density but differed from each other in vine arrangement. In plots of equal density the crop per acre and crop per vine both decreased as vine distance decreased.

different except for the closest vine distance of 2 × 18, which were from 20 to 30 per cent lower than those of the 6 × 6.

This shows that with the strong-growing Black Prince there was no loss of yield per acre by the avenue planting of 6 × 12 instead of 8 × 9 or of 3 × 12 instead of 6 × 6. With Muscat there was a notable increase in each case with the avenue system which, however, is probably due to

the cordon pruning. A further reduction of vine distance to 4×18 caused a loss of about 38 per cent with the Black Prince as compared with the 8×9 plot, but had little effect with the Muscat. A reduction to 2×18, however, caused a loss of 20 per cent with the Black Prince, and 32 per cent with the Muscat, as compared with the 6×6 plots.

Too great a difference between the width of the avenue and the distance between the vines in the row causes a considerable reduction in vine yield and therefore in acre yield. Cordon pruning, at least for several of the first few crops, seems to be useful in neutralizing the reduction within limits which vary with the character of the vine variety.

Figure 4 illustrates the approach to maximum bearing made by the various plots. This maximum, under the conditions of the experiment, is assumed to be the mean crop of the 4×4 Muscat plot in the third and fourth years of bearing, or 15 tons per acre, taken as 100 per cent.

The acre crop of the Black Prince in the 12×12 plot is 54 per cent of the maximum and increases with each increase of density in the square plantings to 91 per cent in the 4×4 plot. The crop of the Muscat with 33 per cent in the 12×12 plot increases in a similar way to the 100 per cent in the 4×4 plot.

A curtailment of the crop due to the uneven distribution of the vines in the avenue systems is shown in virtually all cases and would have been greater if the spur pruning of the square-system plots had been used with the avenue plots.

It is plain that the arrangement of the vines by the avenue system restricts the crop for at least several years and the more seriously, the greater the departure from the square system. With time this restriction diminishes and with a moderate departure from the equal distribution of the square system may disappear. Any extreme departure, however, will in all probability involve a permanent loss of crop.

THE INHERITANCE OF RESISTANCE TO RUST IN THE SNAPDRAGON[1]

S. L. EMSWELLER[2] AND H. A. JONES[3]

INTRODUCTION

THE RUST (*Puccinia antirrhini* D. and H.) of the cultivated snapdragon (*Antirrhinum majus* Linn.), was first observed by Blasdale[2][4] in 1896. For some time thereafter it was apparently confined to the Pacific Coast, but in 1913 it suddenly appeared in the vicinity of Chicago, Illinois, whence it has spread rapidly to all sections of this country, to Mexico, and to Canada. Within recent months it has appeared in England where it will probably become widespread because of the very favorable climatic conditions.

The work of Mains[4] indicates that the rust is heteroecious and that it probably has pycnia and aecia on an alternate host. All his attempts to infect snapdragon plants with germinating teliospores were unsuccessful. He predicts that the alternate host will probably be found in California on native species of *Antirrhinum* in localities where those plants are naturally infected with rust.

When the disease first appeared in the Middle West, florists were unable to control or check it, so that the growing of snapdragons under glass became exceedingly hazardous. The rapid spread of the rust was probably caused by the method of propagation then in use. Many florists had their own strains, which they increased by cuttings from a desirable plant. The shade and high moisture conditions of the cutting bench afforded ideal conditions for rust, and interstate shipments of rooted cuttings probably caused its wide distribution.

Peltier[6] demonstrated clearly that the disease is not seed-borne. This fact stimulated propagation by seed, which called for varieties more homozygous for type; and in a short time many were developed, while the cutting method of propagation was discontinued. At present, florists can somewhat control the disease under glass by careful regulation of watering, ventilating, and temperatures.

When snapdragons are grown out-of-doors the disease is almost uncontrollable. Since spraying with fungicides has not given satisfactory results, this flower has almost disappeared from the gardens of many sections.

In California, where practically all the American snapdragon seed is produced, the rust presents a grave problem. Frequently the yields amount to only a few pounds per acre, and 75 pounds per acre is very rare. To increase the yields of seed California seedsmen have followed various practices—notably early planting, and the use of land on which snapdragons have not been previously grown.

An early autumn planting usually produces a large, vigorous plant before the rust becomes active in the spring. Such plants flower early and may have some maturing seed by the time the rust appears. Although this practice is probably beneficial in seasons when climatic conditions do not favor the rapid spread of the disease, the writers have seen several such fields very badly infested in early spring.

When snapdragons are planted, according to the second practice, on soil that has not grown a crop previously, they are usually not very heavily infected. If, however, the same land is replanted year after year, the infestation becomes increasingly severe.

The common practice at present is to withhold water from the plants as soon as rust appears. Though undoubtedly unfavorable for plant growth, this precaution is necessary if the spread of the disease is to be retarded.

METHODS AND MATERIALS

In 1929 the writers became interested in trying to control snapdragon rust by means of resistant varieties. They made trips to some of the large flower-seed ranches in California, observing the disease in every field visited. Since diligent search failed to reveal a single plant showing resistance, there are evidently no "escapes" under conditions of severe infestation.

In 1929 several varieties were grown at Davis, California, and individual plants were self-pollinated to determine whether inbred lines vary in their degree of susceptibility. Dr. J. B. Kendrick, of this Station, also supplied some snapdragon seed of resistant selections, obtained

from Dr. E. B. Mains of Indiana, whose work has been described from
time to time in the annual reports of that Station. In 1930 several hun-
dred seedlings were grown from the Indiana seed and from selfed seed
produced at Davis. Seedlings of both lots were planted at Colma in the
San Francisco Bay region, and on the Waller-Franklin flower-seed

TABLE 1

RESULTS SECURED BY SELFING RESISTANT PLANTS IN 1931; POPULATIONS GROWN
IN 1932

Plant No.	Number resistant	Number susceptible	Deviation	$\frac{D}{P.\,E.}$
4	41	14	0.25	0.11
10	66	0	....	....
12	52	0	....	....
23	106	34	1.00	0.28
24	73	27	2.00	0.68
26	187	64	1.75	0.37
103	68	0	....	....
107	51	0	....	....

TABLE 2

RESULTS SECURED BY CROSSING RESISTANT PLANTS WITH COMMERCIAL VARIETIES IN
1931; PROGENIES GROWN IN 1932

Resistant parent	and	susceptible parent	Number of plants	Number resistant	Number susceptible
10	×	Apple Blossom	28	28	0
10	×	Salmon Pink	31	31	0
10	×	Canary Yellow	14	14	0
12	×	Apple Blossom	16	16	0
26	×	Canary Yellow	26	16	10
24	×	Advance	18	7	11

ranch at Guadalupe, California. A number grown from the Indiana
seed showed a very high resistance. Although no plants were completely
free from rust, several had only a few small sori. These resistant indi-
viduals were allowed to open-pollinate, and a large amount of seed was
harvested from each. In 1931 their progenies were grown on the Uni-
versity Farm at Davis, on the Ferry-Morse ranch at Salinas, and on the
Waller-Franklin ranch at Guadalupe. In these large populations were
found several plants that were entirely free from rust. In the fall a few
of the most desirable of the resistant individuals were dug and removed
to Davis, where they were transplanted into a greenhouse bench. In
1931 most of them were self-pollinated, but a few crosses were made to
the commercial varieties Advance, Apple Blossom, Salmon Pink, Canary
Yellow, and Beacon.

The selfed and hybrid seed secured in 1931 was grown in 1932, with the results shown in tables 1 and 2. Not all the data are included in these tables—only sufficient to show that the resistant plants were of two types, one homozygous for resistance and the other heterozygous. The results also indicate that resistance is controlled by a dominant gene.

TABLE 3

RESULTS SECURED IN 1933 FROM SELFING AND FROM CROSSING HOMOZYGOUS AND
HETEROZYGOUS RESISTANT PLANTS WITH SUSCEPTIBLE VARIETIES

Pedigree	Number resistant	Number susceptible
Line 26 (heterozygous resistant) × susceptible variety	2,664	2,587
Line 26 (homozygous resistant) × susceptible variety	392	0
Line 10 × susceptible variety	562	0
Susceptible hybrids, self-pollinated	0	310
Heterozygous resistant plants, self-pollinated	363	137
Susceptible hybrids back-crossed to susceptible varieties	0	415
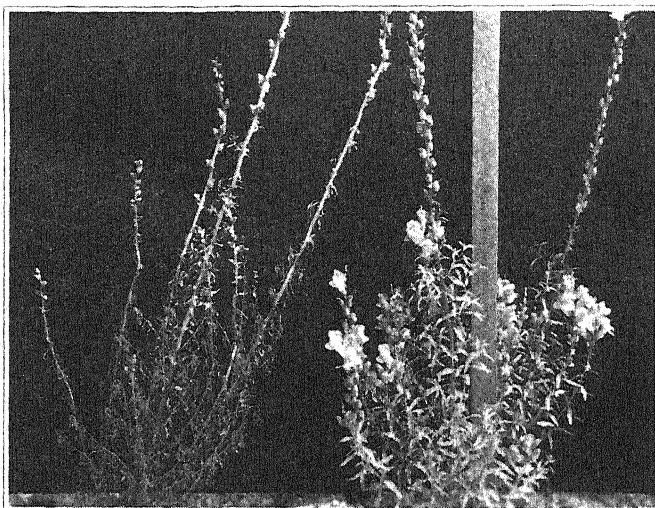
Fig. 1.—A susceptible plant (left) and a resistant plant (right)
growing side by side in the field.

In order to check the results further, additional crosses were made by the authors in 1932 between certain resistant plants and some of the more important commercial varieties. Thirty-two resistant plants of line 26 were used as seed parents in crosses with the standard commercial varieties Brilliant Rose, Harmony, Atro-Coccineum, Fascination, Canary Yellow, Snowflake, Cardinal, Apple Blossom, Red Emperor, and Salmon Pink. The resulting progenies were grown in six localities in

California, with the results shown in table 3. The figures given represent the total counts made in the state in 1933.

Of the 32 plants in line 26, 9 proved to be homozygous for resistance. Progeny from all these plants were grown at Davis, Salinas, Guadalupe, Santa Maria, Lompoc, Pasadena, and El Monte. In all locations their reaction toward the rust organism was the same. The results at El Monte were particularly striking; seed was sown in June, 1932, and the plants were wintered-over in the field. In the early spring susceptible plants began to show lesions, and by May they were either dead or badly infected. Figure 1 shows a resistant and a susceptible plant growing side by side in the field.

TABLE 4

BACK-CROSS PROGENIES DESCENDING FROM RESISTANT PLANTS 16 AND 20

	Partially resistant	Susceptible	Resistant
(16 × Salmon Pink) × Salmon Pink	14	29	15
(20 × Beacon) × Beacon	34	61	17

Crosses were also made between plants from the resistant line, No. 10, and the varieties Cheviot Maid Supreme, Apple Blossom, Red Emperor, Beacon, and Advance—all susceptible. All crosses involving plants from line 10 produced only resistant hybrids; all the back-crosses involving a heterozygous plant gave the expected 1:1 ratio; and those back-crosses and self-pollinations involving susceptible plants gave only susceptible progeny.

MODIFYING FACTORS

Besides the resistant plants two slightly susceptible ones were found in 1931, growing in the progenies of the resistant plants that had been open-pollinated in 1930. These two plants, Nos. 16 and 20, were crossed with commercial varieties, the former to Salmon Pink and the latter to Beacon. From these crosses, only 19 hybrid plants were grown; 10 from the first and 9 from the second. All were planted in the greenhouse. In each lot of hybrids 2 were susceptible, the others being recorded at that time as resistant. In each lot a hybrid which was classified as resistant was back-crossed to its commercial parent in 1932. When these back-cross progenies were examined, three types of plants were found in each population—resistant, slightly susceptible, and susceptible. It is improbable that this situation was the result of the appearance of a second strain of rust, for the three types were found in only two populations, both descending from slightly susceptible plants. These populations

were completely surrounded by other resistant strains, none of which showed this condition.

For this condition no explanation is offered, other than the probable presence of modifying factors. The numbers, though rather small, indicate that an analysis of the situation should not be difficult. It would be very interesting to know whether the factors responsible are carried in commercial varieties. Whenever a plant from line 10 has been used as the resistant parent, it was found to have complete resistance. This statement, however, does not imply that modifying factors are not present in commercial varieties, since only a few of the latter have been used in the crosses.
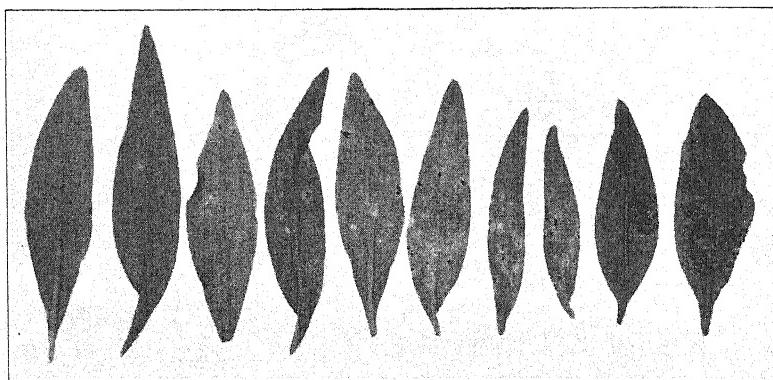
Fig. 2.—Leaves of snapdragons, showing variations from complete resistance to susceptibility.

Until this situation was encountered the origin of the resistant plants found in populations grown from the open-pollinated slightly susceptible plants of 1930 could not be well explained. If modifying factors are present, segregation might be expected eventually to produce plants from which they had been eliminated. The total population of about 5,000 grown in 1930 presented a wide range from complete susceptibility through different degrees of resistance to complete resistance. Only a few plants were of the latter type. Plants 16 and 20 were selected from among the most highly resistant, so the fact that each produced several resistant plants in the back-cross generations is not unusual, if each carried but few of the modifying genes. Since they had susceptible plants, too, in their progeny, they were also probably heterozygous for resistance. Some of the resistant hybrid progeny of plants 16 and 20 must have carried modifying factors even though they showed no rust in the greenhouse, where conditions for rust are not so ideal as in the field, and where very slightly susceptible plants might easily be counted resistant.

Figure 2 shows the various types of resistance found in the two populations derived from plants 16 and 20, together with leaves from completely susceptible specimens. The snapdragon leaves included in this figure, taken from different plants in line 20, represent fairly well the actual range of resistance. Only a few leaves on such plants are attacked, and often a rather thorough examination is necessary to locate any infection whatever. Other resistant plants are rather heavily infected, but never so severely as fully susceptible ones. The leaves depicted display lesions very similar to those on resistant wheat plants shown by Mains and Jackson,[5] whose scale for measuring resistance would be a very interesting method of studying the degrees of resistance in snapdragons. Among the large populations grown in 1931 there were undoubtedly plants showing all types of resistance covered in the scale. In the snapdragon, however, completely resistant plants have not even the light flecking on the leaves, that appears on some wheat plants resistant to leaf rust. This situation is somewhat similar to that reported by Briggs,[3] who found evidence for factors modifying the resistance of wheat to bunt, and who gives a rather detailed discussion of modifying factors in general.

METHODS OF ISOLATING RESISTANT PLANTS

In 1932 it was found that susceptible plants could be infected and eliminated in the early stages of growth. According to the procedure used, plants of lines known to carry resistance were transplanted to 2½-inch pots. After becoming established, some were placed in coldframes, the remainder on a greenhouse bench. Every fourth row consisted of various known susceptible varieties. Thus a check was made upon the efficiency of inoculation under both environments. All were then covered with cheesecloth. On several successive evenings each lot was thoroughly syringed with water and had heavily rusted branches shaken over it. The cheesecloth covering was kept moist for several days and then removed; thereafter, the plants were watered and tended normally. The first sori appeared in about ten days, and within three weeks all the commercial plants were infected, as well as the susceptible ones in the selfed and back-cross populations.

DESCRIPTION OF RESISTANT LINES AND OF HYBRIDS BETWEEN THEM AND COMMERCIAL VARIETIES

All four homozygous resistant strains are undesirable as commercial types. The plants are bushy, profusely branching, each producing numerous slender-stemmed spikes. The flowers are small, spaced far apart on the spike, and the colors are generally mottled. The blooming period

being so late, they must be planted from four to six weeks earlier than standard commercial varieties in order to begin blooming at the same time. Each strain is also somewhat self-sterile and does not set much seed even when open-pollinated, although the pollen and eggs are both func-
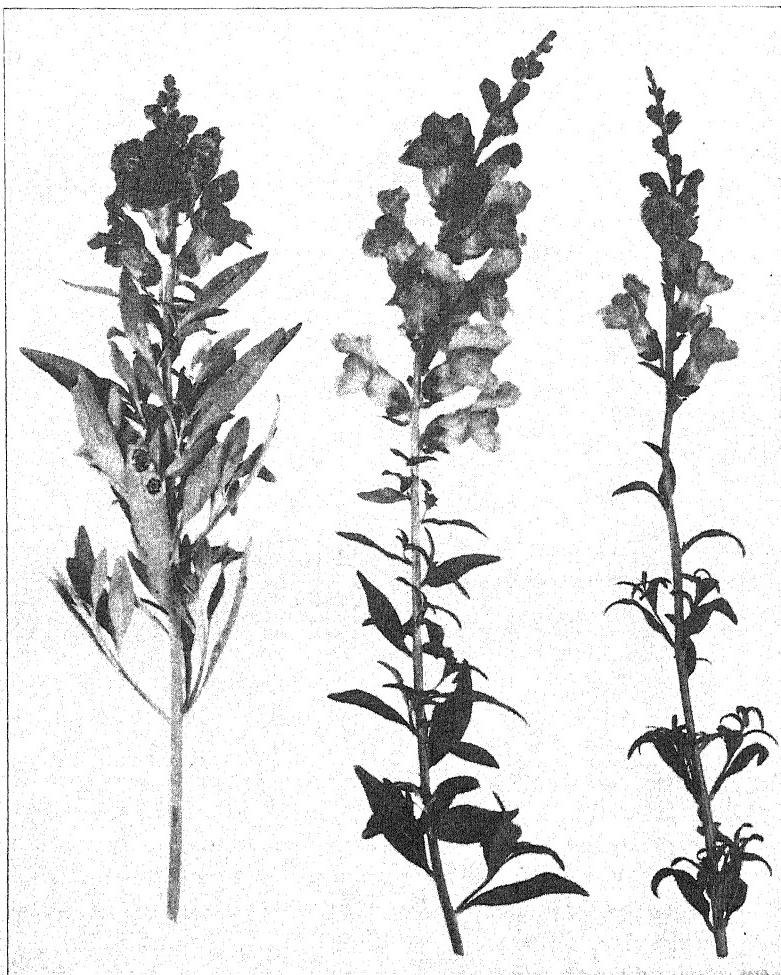

Fig. 3.—Showing inheritance of rust resistance. Left, Red Emperor, susceptible; right, No. 10, resistant; center, F₁, resistant.

tional. These characteristics would practically eliminate these resistant lines from consideration by florists, gardeners, and seedsmen.

Crosses have now been made between resistant types and 15 standard commercial varieties. In all instances the hybrids resemble the commercial parent more closely than the resistant, as shown in figure 3. They

exhibit marked vigor, are highly self-fertile, bloom almost as early, and have practically as large flowers as does the commercial parent. When a tall variety of the maximum type has been used in the cross, the hybrids are usually as tall as the taller parent. Low-growing, bushy varieties, such as Red Emperor and the Majestics, when crossed with line 10 produce an F_1 population with a growth habit practically the same as that of the commercial parent.

When an F_1 hybrid plant, free from modifying genes, is self-pollinated, it segregates (for rust) into a ratio of 3 resistant to 1 susceptible. If a number of these resistant segregates were self-pollinated, one-third of them would breed true for resistance, and two-thirds, or those heterozygous for resistance, would again segregate in a 3:1 ratio. This work does not purport to analyze the inheritance of any characters other than resistance. Plants in F_2 populations have exhibited a wide range of colors and types.

METHOD OF COMBINING RESISTANCE WITH DESIRABLE CHARACTERS

In order to secure acceptable commercial types as rapidly as possible, the back-cross method has been used. The variety Red Emperor, a low, bushy type with large, dark-red flowers, was crossed with line 10, a type slightly taller in growth, very much branched, and bearing smaller flowers of a mottled rose-and-white color. The hybrid plants were all taller than either parent; their flower color was a dark cerise; they were all resistant to rust and bloomed about a week later than the variety Red Emperor. Several of these hybrids were then back-crossed to Red Emperor, and a 1:1 ratio for resistance was secured. The susceptible plants were discarded, and only the resistant individuals that most closely resembled Red Emperor were selected for use in a second back-cross. This procedure of back-crossing the resistant plants to the commercial parent should be carried through a number of generations until the back-cross population shows a very high uniformity with Red Emperor. Then the best resistant plants should be self-pollinated. The progeny will segregate in a 3:1 ratio for resistance. A large number of the resistant plants should then be self-pollinated, and those homozygous for resistance will form the basis for a new resistant type, which should very closely resemble Red Emperor.

The number of back-crosses necessary to produce a line homozygous for flower color, habit of growth, and general morphological characters will probably vary with different varieties, according to the genetic relationship of the colors and other characters. The inheritance of color in snapdragons is known to be very complex, as shown by Baur[1] and by

Wheldale,[7] who have demonstrated the presence of at least 18 different genes affecting color. Various combinations produce complex color patterns, such as color of the tube differing from the rest of the corolla, and various degrees of mosaic color patterns. In order that a homozygous combination may be secured as rapidly as possible, the commercial parent used in the first cross and in subsequent back-crosses should be as nearly homozygous as possible for color and type. With such a parent, from three to five back-crosses should suffice to produce a fairly homozygous population.
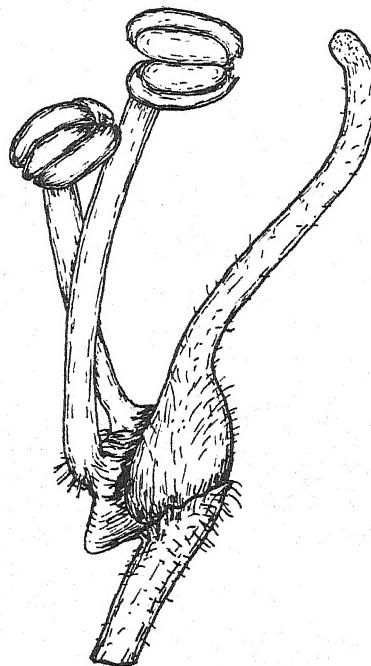
Fig. 4.—Young bud of snapdragon with perianth and two stamens removed.

The procedure described above has been started with the varieties Cheviot Maid Supreme, Autumn Glow, Ceylon Court Yellow, Canary Yellow, Apple Blossom, Red Emperor, Advance, Majestic Red Chief, Majestic Twilight, and Majestic Orange King. First and second back-cross material has already been sent out to California seedsmen. The results so far have been very promising, many resistant plants in the first back-cross generation appearing identical with the commercial parent.

POLLINATION TECHNIQUE

The snapdragon flower is very easy to manipulate for cross-pollination. Emasculation may be performed at any time before dehiscence of the anthers, which does not occur until the buds are large and the flower is

Fig. 5.—Method of bagging for self and cross-pollination.

within a day or two of opening. A young bud at this stage, with the corolla and two stamens removed, appears in figure 4. At this age, when the flower is not readily injured, the stamens may be removed with ease; they are four in number—two long and two short. In the bud stage the filaments are shorter than the style; but they elongate rapidly until, at

dehiscence, the anthers on the longer filaments are in contact with the stigma. The arrangement indicates that self-pollination among snapdragons is common.

When a plant has been selected as a seed parent for a cross, one or more spikes are chosen, and the top of each is pinched out, leaving from 7 to 10 buds. A bag is placed over the spike and fastened to a stake, and a complete record of the cross is placed on a small tag tied below the bag (fig. 5). Emasculation of the buds on each spike may take as long as a week, since the buds progressively mature from the base of the spike to the tip. Pollination is begun as soon as the stigma is receptive—that is, from two to three days after emasculation. Thus both emasculation and pollination may be occurring on a spike at the same time. Pollination is usually accomplished by removing a freshly dehisced anther with a pair of forceps and rubbing the pollen on the stigma. After fertilization, the corolla withers and drops. The seed is harvested as soon as the ovary dehisces, for, if allowed to remain on the plant, it may be partially lost by shattering. The yield will average about 500 seeds per capsule.

RESISTANCE TO RUST IN SOME OF THE OTHER ANTIRRHINUM SPECIES

In 1932 the Division of Foreign Plant Introduction, United States Department of Agriculture, secured seeds of several European species of *Antirrhinum* which were collected by E. Baur (Germany) while on a trip into Spain. In November of 1932, each lot of this seed was planted in the greenhouse at Davis, and in January all were exposed to rust. The seedlings were transplanted to the field in the early spring and were grown in a plot adjacent to susceptible plants of *Antirrhinum majus*. The results of this test appear in table 5.

It is very interesting that resistance to rust should be found in any of the European species. The disease does not occur in Europe, and most likely none of these species have been exposed to this disease at any time during their evolutionary history. Probably, as shown in the table, some were homozygous for resistance, some heterozygous, and others all susceptible. The degree of susceptibility varied considerably, some being attacked very lightly, while others were killed. In all instances the resistance was complete, not a single sorus being found on any of these plants.

Several of the species resembled very closely some of the resistant plants derived from the seed sent by Dr. Mains. The flowers were about the same size, the leaves similar, and the plants also highly self sterile. No attempt has been made by the authors to determine the nature of the

resistance in these plants, but most likely the resistant gene secured from the Indiana material had its origin in some of these European species.

The most generally interesting part of this phase of the work is the demonstration of the great potentialities in the field of foreign plant introduction. Very probably, resistance to many diseases of our economic crop plants could be discovered in closely related forms or species growing in other parts of the world. The desired character, even though

TABLE 5

REACTION OF SEVERAL EUROPEAN SPECIES* OF ANTIRRHINUM TO RUST

Number	Species of *Antirrhinum*	Number of resistant plants	Number of susceptible plants
136	*glutinosum* (Capileira)	32	0
137	*glutinosum* (Orgiva)	0	28
138	*hispanicum* (Celorico)	3	12
139	*ibanjezii* (Cartagena)	16	0
140	*molle* (Lerida)	0	21
141	*molle* (Monsech)	18	0
142	*siculum*	24	0
143	*tortuosum*	0	29
144	species ? (Chorro)	3	8
145	species ? (Cintra)	0	16
146	species ? (Lucena)	10	12
147	species ? (Zaragoza)	17	0

* These species were not determined by the authors, but are here published, with locality names, as listed by E. Baur.

found in another species of the genus, may possibly be incorporated with the more desirable characters of our economic plant. The sanctity of species delimitation is slowly fading as reports of synthetic species formation continue to accumulate.

SUMMARY

The rust (*Puccinia antirrhini* D. and H.), of the cultivated snapdragon (*Antirrhinum majus* Linn.), was first observed in California in 1896. In 1913 the disease appeared in the vicinity of Chicago, Illinois; and since then it has spread to practically all parts of the United States.

Methods of control have not proved entirely satisfactory, particularly when snapdragons are grown out-of-doors. Under greenhouse conditions, the disease can be somewhat checked by certain cultural practices.

In 1929 unsuccessful attempts were made to find resistant plants in the seed fields in California. The next year seed was secured from resistant selections made at the Indiana Experiment Station, and large

populations were grown in several localities in California where rust was particularly severe.

Several plants found in the population from the Indiana seed showed a very high resistance to the disease. These were open-pollinated, and a large amount of seed was harvested from each.

In 1931 progenies from these highly resistant plants were grown in various locations in California, and a very few plants from each location were entirely free from rust. A few of these resistant individuals were removed to Davis, where they were self-pollinated and crossed with several known susceptible varieties. The results showed that resistance is controlled by a single dominant gene.

Several highly resistant plants were used in crosses with known susceptible varieties, and the results indicated the presence of modifying genes. This seems to be a logical explanation for the fact that immune plants were secured from highly resistant parents, since segregation would tend to produce some types free from modifying genes.

The original plants are undesirable types, used only because of their resistance. To transfer the resistant gene to good commercial varieties as rapidly as possible, the back-cross method has been utilized. Progress so far has been very encouraging.

ACKNOWLEDGMENTS

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THRIPS RESISTANCE IN THE ONION

H. A. JONES, S. F. BAILEY, AND S. L. EMSWELLER

THRIPS RESISTANCE IN THE ONION[1]

H. A. JONES,[2] S. F. BAILEY,[3] AND S. L. EMSWELLER[4]

INTRODUCTION

THE SELECTION and breeding of plants resistant to parasites had its inception chiefly in the field of plant pathology, more specifically in the development of cereals resistant to rust. While the breeding for resistance to insects is still in its infancy, the possibilities in this field appear to be almost unlimited. In certain cases, among which may be mentioned the control of onion thrips, breeding for resistance seems to offer promise. In this paper are presented data which show that in the case of the onion certain varieties do possess a definite resistance to thrips, and the characters thought to be responsible for this resistance are described in some detail.

Howitt,[22][5] McColloch,[30] Martin,[29] and others have given excellent general reviews of the development of resistant crop plants; here only the more important papers concerned with resistance to sucking insects are reviewed.

The causes of resistance to insects have been grouped by Wardle and Buckle,[43] McColloch,[30] and Wardle[42] as physical, chemical, or physiological. The first category includes such characters as hairiness, thickness of epidermis, thickness of seed coat and rind, and habit of growth; the second, the presence of such compounds as acids, alkaloids, essential oils, and tannin together with the potash-phosphoric acid ratio; the third, such characters as vigor, seasonal adaptation, early maturity, ability to recover from injury, and positive or negative response to specific stimuli. In most instances, however, the characters, whether physical (morphological), chemical, or physiological, are probably genetic in nature and are therefore governed by the laws of inheritance. Resistance may result from one character, or from several combined; and the effectiveness of a character may vary with the soil condition and climate.

Among the physical or morphological characters that seem to be intimately associated with host resistance is hairiness. Hollowell, et al,[21] state that the English and Italian types of red clover, which are gla-

brous, suffer much more from the potato leafhopper, *Empoasca fabae* (Harris), than do the American strains, which are hairy. Pieters[34] maintained that the leafhopper was responsible for the development of the hairy American strain. This insect, native to the United States, has probably hindered the reproduction of the more glabrous plants so that only the hairy strains have survived. Fenton and Hartzell[12] thought that the hatching nymphs became entangled in the hairs, whereas Monteith and Hollowell[31] suggested that some character in addition to hairiness may be involved. About the same time Poos[35] found that, in the seedling stages, all varieties of red clover succumbed when injured by this leafhopper; in addition he observed no difference in the amount of injury to Kansas alfalfa and the more hairy Peruvian type. Poos and Smith,[36] and Jewett[23] also concluded that characters other than the amount and type of pubescence are, at least in part, responsible for resistance. Jewett[24] showed that more force is necessary to penetrate the leaf of the Kentucky than other varieties of red clover, a fact which may account in part for its being more resistant than the Italian. A report from South Africa by Worrall[45] stated that the hairy American Upland cotton is more resistant to the jassid, *Chlorita fascialis* Jacobi, than the more glabrous Sea Island and Egyptian varieties. The hairy types are sufficiently resistant to allow the plants to mature the bolls.

At the Kansas Agricultural Experiment Station, 100 species of grasses, comprising about 80 per cent of the native prairie grasses, were tested by Hayes and Johnston[20] for resistance to chinch-bug injury. The native, perennial species with harsh tissues (*Andropogon scoparious* in particular) proved best able to survive injury and recover.

Mumford,[33] discussing the curly-top disease of sugar beets, suggested that the thicker epidermis and cuticle in the resistant strain may indicate some external protection from the beet leafhopper, *Eutettix tenellus* (Baker). In studying the onion thrips on cotton, Wardle and Simpson[44] noted that the underside of the leaves is preferred, apparently because of a difference in epidermal thickness. Bailey[2] observed the same preference on the part of the bean thrips, *Hercothrips fasciatus* (Perg.), on its native hosts.

Staniland[40, 41] found the Northern Spy apple practically resistant to the woolly aphid (*Eriosoma lanigerum* Haus.), but the same degree of resistance did not exist in roots and branches. According to the evidence, the resistance of apple stocks aboveground depends somewhat upon a high per cent of sclerenchyma encircling the stem and preventing penetration. The middle lamella, however, can be dissolved by the saliva of the aphid, so that the check formed by the sclerenchyma may be overcome eventually. He concluded that resistance cannot be ex-

plained wholly by mechanical considerations. *Aphis rumicis* Linn. was found on stocks resistant to woolly aphid. Apparently, then, resistance to these two aphids is associated with different characters. Davidson[8] reported that the saliva of *A. rumicis* can dissolve a passage for the piercing organ but that the presence of a thick cuticle may prevent the *young* aphid from piercing certain tissues. Monzen[32] in Japan thought that a greater pH concentration in the sap or a "specific repellent ingredient" caused resistance of apple stocks to the woolly aphid. Salman[37] considered the Zuccalmaglio-Reinette variety of apple to be resistant to attacks of woolly aphids. Resistance was most noticeable in grafted trees, the stock being infested while the scion was comparatively free. Lepelley,[27] conducting some tests with seedlings derived from crossing the Northern Spy with susceptible varieties, concluded that this variety was heterozygous for resistance.

Even less clearly understood are the chemical factors that are thought in some way to control resistance. Comes[6] stated that acidity of the plant sap caused by organic acids afforded protection and that malic was considered the most toxic. Gernert[15] stated that F_1 hybrids of teosinte and yellow dent corn resembled teosinte and were resistant to attacks of *Anuraphis maidi-radicis* (Forbes) and *Aphis maidis* Fitch. The tougher leaves and more bitter sap of the teosinte parent and the hybrid probably account for their being more resistant than corn.

Andrews[1] showed that a high ratio of potash to phosphoric acid in the tea plant acted inimically to *Helopeltis theivora* Waterh. (a mirid). Though the normal ratio (potash to phosphoric acid) is about 2 to 1, the resistant plants had a ratio of 4 to 1. Attempts to increase the normal ratio gave variable results. Direct injection of potash was not effective. The results of Dementiev[11] on the control of woolly aphid after the injection of barium chloride (1 to 350) into the roots of apple trees were variable. Sanford[38] controlled cottony cushion scale (*Icerya purchasi* Maskell) on Spanish broom by filling with potassium cyanide crystals a hole bored in the trunk.

Although the field of physiological resistance is exceedingly complex, we may well discuss some of the scattered references in the literature.

Harland[16] found that certain native Indian strains of cotton were resistant to leaf-blister mite, *Eriophyes gossypii* Banks. Flint and Hackleman[13] observed that Champion White Pearl, Democrat, and Black Hawk varieties of corn were able to grow vigorously under the same infestation of chinch bugs that killed the more susceptible varieties. Davidson[7] found that the eighteen varieties of *Vicia faba* that he studied were much more susceptible to *Aphis rumicis* than is *V. narbonensis*, the probable prototype of *V. faba*. Resistance seemed to be associated

with the "general physiology of the plant." Searls[39] observed that the degree of aphid infestation (*Illinoia pisi* Kalt.) was less severe on the yellow than on the greener plants or varieties of peas. The same held for alfalfa and sweet clover infested with *Empoasca fabae* Harris.

According to Lees,[26] a mite (no species mentioned) that infests currants and normally feeds on the products of the hypertrophied tissue (the plant's response to the wound stimulus) cannot maintain itself on the red currant *Ribes vulgare,* which develops no hypertrophied tissue. Seabrook's Black, a variety of *Ribes nigrum,* is resistant because the mite kills the growing point and thus starves itself. Harland,[18] reporting that certain varieties of cotton are resistant to the leaf-blister mite (*Eriophyes gossypii* Banks), suggests that resistance results from lack of the gall formation that occurs in susceptible varieties.

In many instances the nature of resistance has not been suggested. Such a case is that of the grape phylloxera, among the first to receive consideration because of its great practical importance. Certain species indigenous to the Mississippi Valley were found to be resistant and have been used successfully as root stocks in infested regions. This subject has been thoroughly discussed by Davidson and Nougaret,[9] Bioletti, *et al,*[4] and Börner.[5]

Beach and Maney[3] secured resistant hybrids by crossing the sand cherry, *Prunus besseyi* Bailey, which is resistant to aphids (no species given), with Montmorency cherry (*Prunus cerasus*) and with Wyant plum (*Prunus americana*), both of which are susceptible. Resistance was found to be inherited as a simple dominant, but its nature was not determined.

Harland[17] observed that two types of Seredo cotton were resistant to the black scale (*Saissetia nigra* Nietn.), but did not suggest the cause of resistance.

Wardle and Buckle[43] stated that the Leconte and Kieffer varieties of *Pyrus,* which are F_1 hybrids between the Chinese pear (*Pyrus sinensis*), resistant to San Jose scale, and the susceptible *Pyrus communis,* resemble *P. sinensis* in resistance.

In none of the cases cited above has the exact nature of resistance been determined. With such a complex condition presenting itself, detailed and highly technical experiments must precede any definite conclusions regarding the exact nature of resistance. Obviously, too, even though the feeding process in the sucking insects mentioned is very similar, the characters causing resistance are not at all comparable, and each problem must be treated individually.

STUDIES ON RESISTANCE TO THRIPS

For three-quarters of a century or more, investigators have sought a satisfactory method of controlling thrips (*Thrips tabaci* Lind) on the onion (*Allium cepa* L.). Although thrips are readily killed by various contact insecticides, usually a number of rather costly applications are necessary. Satisfactory chemical control, indeed, has thus far been impossible for several reasons: A large number of thrips are always protected between the inner leaves of the onion plant, the pupal stage is spent in the soil, the species is very prolific, the generations overlap, natural parasites are lacking, and other host plants are numerous. The enormous damage to the onion crop in California and the unsatisfactoriness of chemical control have necessitated a mode of attack different from that made in the past upon this insect.

Growers who have compared different varieties planted side by side have observed that the Spanish types are somewhat more resistant to thrips injury than are such varieties as Australian Brown and Southport Yellow Globe. The Spanish types do suffer less under conditions of moderate infestation than the so-called American types; but under conditions of extreme infestation as occurred in Davis, California, in 1931 they also were killed prematurely. This difference in the susceptibility of onion varieties suggested the possibility of developing resistant ones, and this work has now been under way for several years at the California Agricultural Experiment Station.

Throughout this investigation, the Division of Foreign Plant Introduction of the United States Department of Agriculture has closely coöperated with the present authors. It has secured seed from many countries, which were tested in the breeding plots at Davis, along with most of the varieties commonly grown in this country. Of chief interest were the introductions from countries of western Asia, especially the area extending from Palestine to India which De Candolle[10] assigned as the probable native home of the onion. In this region, then, we should expect to find the greatest diversity of form, and perhaps a variety with a high degree of resistance.

Comparison of Thrips Population on Different Types of Onions.—In a study of thrips resistance MacLeod[28] in New York State, classified varieties of onions as susceptible, average, and resistant, according to the number of thrips present on the plant. As susceptible, he lists Southport Red Globe, Extra Early Barletta, Red Wethersfield, Mountain Danvers, Ebenezer, and Yellow Globe Danvers; as average, Crystal White Wax, Yellow Strasburg, Prizetaker, and Southport White Globe; as resistant, Utah Valencia, Utah White Sweet Spanish, Valencia, Riverside

Sweet Spanish, Sweet Spanish, Extra Early Red Flat, White Portugal, and Yellow Danvers Flat. The results secured at Davis have not coincided exactly with these. The last three varieties, especially, cannot be classified as resistant here, either as to number of thrips or as to freedom from injury (table 2).


Fig. 1.—The three rows in the foreground to the left are the variety White Persian; the others are Australian Brown. Note the serious damage done by thrips to the latter variety and the freedom of the White Persian from injury. Photographed, June 26, 1931.

In 1931, at Davis, occurred an exceptional opportunity to observe the resistance to thrips of a number of domestic onion varieties and foreign introductions. As conditions were ideal for the rapid increase of thrips, infestation was very severe, and most of the varieties were killed early in the season. The leaves dried from the tips down, causing the premature death of the plants. One introduction, however, FPI 86279 from Persia (fig. 1), remained green throughout the season and showed no injury. This variety, which is here called White Persian, was outstanding in its

resistance. The Spanish types, while showing somewhat less injury than the American, were also killed prematurely.

In 1932 a daily count of the thrips population was made on several varieties (table 1) from May 11 to June 1. In some instances there were only a few plants available for observation. The data, being very interesting, are included here; they agree closely with the results obtained in 1933 (table 2).

In 1933 a more comprehensive test of thrips resistance was made. This included varieties and strains of onions commercially important in the

TABLE 1

VARIETIES OF ONIONS ARRANGED ACCORDING TO THE NUMBER OF THRIPS (ADULTS
AND LARVAE) PER PLANT; DAVIS, CALIFORNIA, MAY 11
TO JUNE 1, 1932

Variety	Number of plants	Average number of thrips
White Persian	16	8.0
California Early Red (21-22-1)	38	20.3
Early Grano	4	26.5
Sweet Spanish	68	29.3
Denia	6	31.1
Australian Brown	28	33.2
Italian Red	26	38.3
Yellow Danvers Flat	4	39.2
Red Wethersfield	12	40.0
Southport Red Globe	24	42.0

United States, as well as all the foreign introductions. The seed was sown in the coldframe the last of November. The seedlings were transplanted to the field on March 29 and were spaced 3 inches in rows 27 feet long and 18 inches apart. A plot consisted of ten plants of a strain, and these were replicated five times (except FPI 101113 and FPI 101533). Irrigation water was applied in furrows between the rows. The plants were not treated with insecticides.

In 1933 only the larvae present were counted. These represent rather accurately a definite proportion of the entire thrips population, which is composed of overlapping generations. They cannot fly, are not difficult to count, and remain on the same plant throughout the larval stage.

At each count, the mean number of larvae per plant was determined for each lot of ten plants. These averages were used to determine the frequency distribution.

Counts were begun on May 9 and were repeated at five-day intervals on each strain, until the first plant matured. A plant was considered mature when the top fell. Counts on Nebuka (*Allium fistulosum*) or

Japanese onion were stopped on July 8, although this is a perennial and continues to grow as long as conditions are congenial. The varieties in

TABLE 2

Varieties and Strains of Onions Arranged According to the Average Number of Thrips Larvae per Plant; Davis, California, 1933

Variety	Mean number of larvae per plant	Period of counting, May 9 to date indicated
White Persian	4.14±0.09	July 8
Nebuka (37-1-1)	5.99±0.23	July 8
39-4	6.43±0.16	June 23
FPI 101460; Poona, India	6.68±0.20	June 13
FPI 101499; Poona, India	6.85±0.21	June 13
44-2	6.99±0.20	June 28
Yellow Bermuda	7.00±0.24	June 13
California Early Red (21-22-1)	7.03±0.19	June 23
Australian Brown (5-317-5); light-green foliage	7.06±0.15	July 13
Crystal White Wax	7.23±0.26	June 13
FPI 101461; Poona, India	7.25±0.26	June 13
Italian Red (13-20-3)	7.39±0.16	June 28
Early Grano	7.66±0.18	June 13
Southport White Globe	7.74±0.17	June 18
Prizetaker	7.86±0.18	June 23
Sweet Spanish	8.14±0.16	June 28
White Sweet Spanish	8.16±0.19	June 23
51-3	8.41±0.25	June 28
FPI 101113; Nanking, China	9.08±0.29	June 28
FPI 101112; Nanking, China	9.17±0.24	June 18
FPI 101533; Burma, India	9.28±0.43	June 23
FPI 101575; Pashawar, India	9.38±0.34	June 18
41-8	9.40±0.35	June 18
FPI 101171; Pusa, India	9.41±0.32	June 13
Extra Early Yellow	9.55±0.19	June 18
Australian Brown (commercial)	9.65±0.24	June 23
42-8	9.78±0.24	July 3
White Portugal	9.91±0.31	June 18
Yellow Strasburg	10.02±0.29	June 18
Extra Early Red Flat	10.32±0.28	June 18
FPI 101224; Punjab, India	10.35±0.28	June 23
Australian Brown (5-222)	10.39±0.25	June 23
Mt. Danvers	10.40±0.29	June 18
Yellow Danvers Flat	10.54±0.30	June 23
Red Wethersfield	11.05±0.29	June 23
Ohio Yellow Globe	11.17±0.26	June 23
Ebenezer	11.35±0.25	June 28
Australian Brown (combined pure lines)	11.60±0.25	June 28
Southport Red Globe	11.69±0.26	June 28
Yellow Globe Danvers	12.05±0.26	June 23
Australian Brown (5-16)	12.62±0.31	June 28
Yellow Bottleneck	12.76±0.39	June 28
Australian Brown (5-24)	12.84±0.33	June 23
Southport Yellow Globe	12.90±0.36	June 23

table 2 that bear numbers only, such as 39–4 and 44–2, are selections from foreign introductions that have been selfed for one generation. Of the introductions listed in the table, only White Persian had characters that would make its further propagation desirable.

All varieties were compared with the White Persian, on which the lowest mean number of larvae per plant (4.14) were found. The difference between this number and that of all other varieties and strains is significant. The varieties fall in practically the same order in 1933 (table 2) as in 1932 (table 1), so that certain of their characters evidently in-
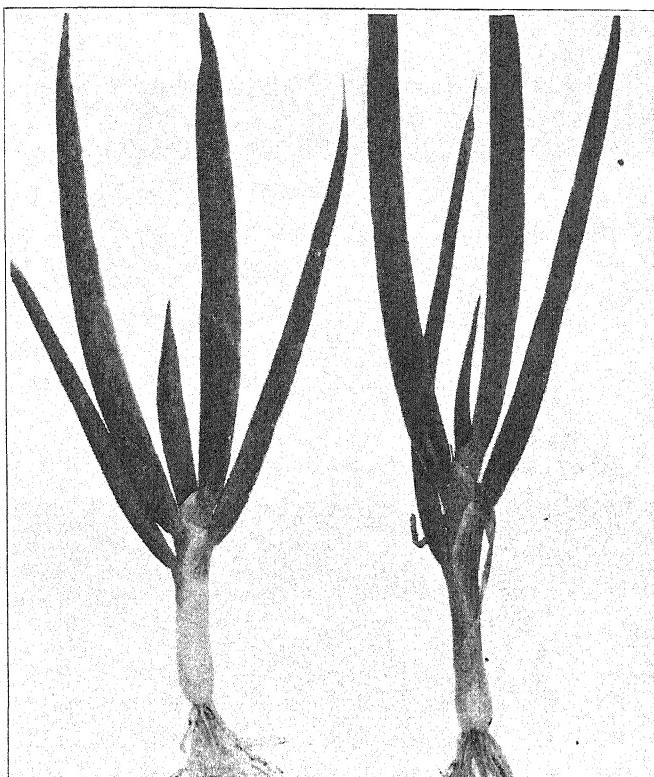

Fig. 2.—Nebuka (*Allium fistulosum*) or Japanese onion. The habit of leaf growth in this species helps to restrict the thrips population.

fluenced the size of the thrips population. The Nebuka, figure 2, which has foliage somewhat like that of White Persian has a thrips population nearly as low, but its leaf tissue is much more severely injured where the thrips have fed.

Most of the Australian Brown strains are severely injured by thrips and, as shown by table 2, support a large population. There is, however, one exception—namely, Australian Brown 5–317–5, which has been inbred for two generations and has foliage similar in color to that of Sweet Spanish.

Nature of Resistance in the White Persian.—The resistance of the White Persian to thrips seems to be determined by two groups of factors: one, probably, controls those characters that hold the thrips population to a minimum; the other helps the plant to withstand injury. Two, or perhaps three, characters apparently tend to restrict the thrips popu-
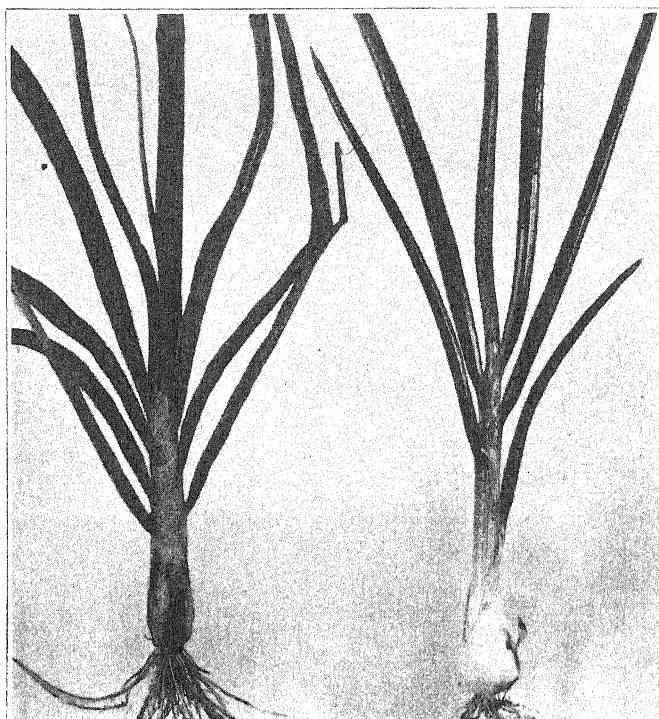
Fig. 3.—Australian Brown on the left; White Persian on the right. Note the habit of growth.

lation—namely, the shape of the leaves, the angle of divergence of the two innermost leaves, and the distance apart of the leaf blades on the sheath column. Probably of considerable importance is the difference in shape of the leaves. In most varieties the leaf blades have a flat side; these sides are face to face and, in the young leaves, closely appressed, protecting the larvae against insect enemies and adverse weather conditions. In White Persian the leaves are almost circular in cross section (fig. 3), reducing protection to a minimum.

The leaf blades of ten White Persian plants were pulled together and tied in order to increase the amount of contiguous leaf surface and thus determine whether the greater protective area formed would cause an

increase in the thrips population. Ten plants of the Denia variety were
handled in the same manner. Ten other plants of each variety not tied,
were used as checks. Before tying and at about seven-day intervals there-
after, the number of larvae per plant was determined. The counts are
presented in table 3.

The marked increase in the number of thrips on the plants whose tops
were tied shows that closely bunched leaves make a very favorable en-
vironment for this insect. This also indicates that resistance in the White
Persian is probably not caused by some toxic component within the
plant, since the percentage increase in the number of larvae per plant

TABLE 3

MEAN NUMBER OF LARVAE PER PLANT WITH FOLIAGE TIED AND UNTIED; 1932

	Number of thrips on date tied, July 14	Number of thrips after		
		8 days	15 days	21 days
Denia, leaf blades tied	78.7	139.3	122.8	37.4
Denia, leaf blades not tied (check)	115.0	81.5	44.8	16.8
White Persian, leaf blades tied	19.3	60.5	80.4	20.3
White Persian, leaf blades not tied (check)	26.1	25.7	3.6	0.9

after the leaves were tied was more rapid in White Persian than in
Denia. The later downward trend of the population was caused by the
maturing of the plants.

The wide angle between the two innermost emerged leaves (fig. 3),
especially in the young plant, is another White Persian character that
helps to restrict the thrips population by reducing the protective en-
vironment to a minimum. Still another character, probably of some im-
portance, is the greater vertical distance between the leaf blades. Each
new leaf extends its sheath farther beyond the one encircling it than in
other commonly cultivated varieties (fig. 3). This habit of growth pro-
duces an extremely long sheath column. If commercial varieties of
onions had these leaf characters, one might secure a more efficient con-
trol by spraying or dusting than at present, because practically all of
the foliage could be covered.

As stated above, the shape and habit of leaf growth in the White Per-
sian probably help to restrict the number of thrips. Other characters
help the plants to withstand injury, but these are as yet not well under-
stood.

As has often been observed, thrips injury becomes most conspicuous
following the first hot days of summer when there is a desiccation and
dying back of the foliage; but it is not known just how high temperatures

accentuate thrips injury. This typical injury is most prominent in varieties with dark-green foliage; less so in the Spanish types which have lighter-green foliage, and is apparently absent in White Persian which has foliage that is even lighter green than that of the Spanish types. Leaf color may be a factor in resistance to injury, because the temperature in the White Persian leaf tissues is possibly lower than in those varieties having darker-green foliage. Similarly, in the tomato fruit, Harvey[19] found dark-green areas to be more subject to injury by sunscald than light-green areas, because of the greater absorption of light with a consequent higher temperature in these areas.

In the White Persian one can determine the exact location where the thrips have fed because these areas are somewhat lighter green in color than the surrounding tissue but they do not seem to dry out. As a thicker leaf tissue might conceivably help to prevent desiccation of the cells surrounding the injured areas, measurements were made to find whether there was a difference between leaves of varieties showing different degrees of resistance. The varieties used were White Persian, Sweet Spanish, and Australian Brown. Sections were taken of the entire circumference at the widest part of mature leaf blades. These were killed, sectioned, stained, and made into permanent slides in the usual manner. The image of the section was projected with a Zeiss microprojector upon a screen, and at each bundle the thickness of the tissue was measured. Leaves of about fifty plants of each variety were so studied. If the thickness of the Australian Brown is taken as 1, then the Sweet Spanish has a value of 1.13; the White Persian 1.32. Analyzed statistically, the differences between the mean thickness of the Australian Brown and White Persian, and Sweet Spanish and White Persian were found to be significant. The difference between Australian Brown and Sweet Spanish may, however, be unimportant. Leaf thickness alone probably does not account for resistance to injury, because certain White Persian plants had leaves about the thickness of Australian Brown but still without typical injury.

History of the White Persian.—The White Persian variety of onion was obtained by Dr. W. E. Whitehouse of the United States Department of Agriculture, Division of Foreign Plant Introduction, in 1929, while he and H. L. Westover were traveling through Europe and Asia Minor to study and collect the forage, fruit, and vegetable crops indigenous to that part of the world. A number of varieties of onions were secured in Persia; but there, according to Dr. Whitehouse, the peasants considered the best onion to be a large white one grown under irrigation in Kashan, a village situated in the hills about 3,000 feet above sea level. Seed of this variety was distributed from Washington as FPI 86279. It was

later named White Persian by the California Agricultural Experiment Station.

Characteristics of the White Persian.—Three crops of White Persian bulbs and two crops of the seed have now been grown at Davis. Although selections have been made within the variety for certain leaf characters, very little attention has been given to the isolation of desirable bulbs. Probably the best use of this variety at present is to cross it with the existing important commercial types, thus incorporating in them the resistant characters.

The chief objections to the variety as a commercial onion are its tendency to split badly and its poor keeping quality. Its strong tendency to bolt is no worse than that of some other varieties. When seeded in cold-frames on September 4, 1931, and transplanted to the field on December 17, about 98 per cent of the plants bolted the following spring. Very little bolting occurred when seeding was done in the coldframe in late November and the transplanting was done in March.

As stated previously, the foliage is of a lighter green than that of any other variety ever grown in the plots at Davis. The innermost leaf, as it emerges, bends away somewhat from the next oldest leaf; and the sheath of the new leaf extends considerably beyond the one encircling it, causing the mature onion to have a conspicuously long sheath column. The leaf blades of the young plant are round in cross section, but those that arise later are more flattened.

The plants mature very late. The bulbs are white and oblate. The flavor is exceptionally mild—probably milder than that of any of the commercial varieties now grown in this country—and unlike that of any other onion variety known to us.

Breeding for Resistance.—An effort is now being made to incorporate the resistance characters of White Persian into susceptible varieties. At present, F_2 and first back-cross generations are being grown in the field and in the greenhouse. In order to expedite the work, the plants are being handled so that one generation is grown each year. If the seed is sown in July, the plants have a longer growing season, and most of them go to seed the following spring.

The first crosses between White Persian and other varieties were made by intermingling under muslin cages. Flies were introduced to do the pollinating, as described elsewhere (Jones and Emsweller[25]). Seed from these White Persian parents was sown in the coldframe; and the F_1 plants having a darker foliage color could be selected from among the seedlings. Some of them were selfed; others were back-crossed to commercial types. Selfing was accomplished by enclosing each umbel within a one-pound manila paper bag; back-crossing, by emasculating

an inflorescence of the plant to be used as the female parent, tying to it one from the pollen parent, and enclosing both within a small cheese-cloth cage. In some cases the plants that were to be crossed were grown side by side (fig. 4). In other cases the flower stem of the pollen parent was severed near the base and placed in a bottle of water, where it re-

Fig. 4.—The two small cages in the foreground are made of a wire frame-work and covered with cheesecloth. Flower heads of the male and female parents are enclosed within, and flies are added to carry on pollination.

mained fresh and produced pollen for more than a week. Flies, hatched artificially, were placed within the small cages enclosing the two umbels; and a set of from 700 to 800 hybrid seeds was often secured. Crossing by means of flies is much more efficient than crossing by hand.

As stated previously, the bulbs are very poor keepers; as a rule they rot and sprout soon after being placed in storage. If set in the field, they usually make a good growth until about the time the plants start to

bloom, but often die from various bulb rots before much of the seed is mature. Fair results have been secured by disinfecting the bulbs shortly after they have been harvested and then planting them in sterile soil before they begin to sprout. This method is used only for plants that are needed for breeding. For the production of large quantities of seed, the plants are not permitted to bulb. If seeding is done in July or in early August, the plants become large and are then set in the field some time during the winter. At Davis these plants produce a good yield of seed and remain in a healthy condition until practically all the seed is mature.

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ORNAMENTAL FLOWERING PLANTS NATURALLY INFECTED WITH CURLY-TOP AND ASTER-YELLOWS VIRUSES[1]

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INTRODUCTION

A FEW OF THE VIRUS DISEASES have been experimentally transmitted to many species of plants in different genera of many families. The host range of virus diseases as determined by experimental infection often does not coincide with the food and breeding plants preferred by the insect vector of the disease under natural conditions: preferred food and breeding plants of the insect under field conditions are sometimes immune to virus diseases; and the insect cannot live for more than a few days in captivity on some host plants that are highly susceptible to virus diseases in the natural environment. A review of the literature on the host range of certain virus diseases as determined by experimental and natural infection follows.

Kunkel[8, 9] reported that he experimentally transmitted aster yellows with *Cicadula divisa* Uhl. to 170 species in 150 genera belonging to 38 families. He transmitted the disease to asters from 40 different species of plants experimentally infected with yellows. There is no record of the natural occurrence of yellows on most of the plants experimentally infected with the disease. Three methods were used by him in determining whether a plant was naturally infected with yellows. In the first method the virus was recovered by previously noninfective leafhoppers from the naturally infected plants and transferred to asters. He proved by this

method that 9 species in 9 genera of 4 families were infected with yellows in the natural environment. In the second method a comparison was made of the symptoms on naturally and experimentally infected plants, but this method does not definitely prove that the disease observed on any yellowed plant is actually aster yellows. He records 12 species in 11 genera of 5 families with this method. The third method used was by grafting yellowed buds into healthy plants. This method was used with tomato plants. The virus was not transmitted to tomato by *Cicadula divisa.*

Priode[11] inoculated 20 different species of plants with the juice from tobacco plants infected with American ringspot and transmitted the disease to only 4 species in 4 genera of 3 families.

Wingard[22] transmitted American ringspot disease of tobacco *(Nicotiana tabacum)* to 62 species in 38 genera belonging to 17 families of plants with several methods of inoculation.

The natural host range of American ringspot includes a total of 11 species in 9 genera of 3 families as determined by Fromme and Wingard,[4] Wingard and Godkin,[23] Wingard,[22] Johnson,[7] and Henderson.[5, 6]

Bald and Samuel[1] experimentally infected with spotted wilt, 35 species of plants in 15 genera belonging to 4 families. The virus was transmitted by the thrips, *Frankliniella insularis,* to 15 species of plants in 10 genera. The virus was recovered by previously noninfective thrips from 6 species of infected plants in 3 genera, and transferred to tomatoes. Thirty-four species in 15 genera were infected with the juice from infected tomatoes by mechanical inoculation. The virus was returned to tomatoes from 30 infected species in 15 genera by mechanical inoculation.

Smith[19, 20, 21] experimentally infected 28 species of plants in 13 genera belonging to 4 families with the virus of spotted wilt. The virus of spotted wilt in the British Isles is not identical with the virus of ringspot described by Wingard and others in the United States.

The natural host range of spotted wilt includes a total of 8 species in 8 genera belonging to 5 families as determined by Bald and Samuel[1] and Smith.[21]

Severin and his coworkers[12, 13, 15, 17, 18] reported that 166 species of plants, in 114 genera, belonging to 36 families were experimentally infected by the beet leafhopper, *Eutettix tenellus* (Baker). Fifty-three species of plants in 38 genera of 16 families were proved to be naturally infected with curly top. A total of 174 species of plants in 115 genera in 37 families were either experimentally infected or demonstrated to be naturally infected with curly top. The virus was recovered from the ex-

perimentally or naturally infected plants by previously noninfective beet leafhoppers and transferred to sugar beets.

McKay[10] found that zinnia and strawflower were susceptible to curly top under laboratory conditions and came to the conclusion, on circumstantial evidence, that these ornamental flowering plants were naturally infected with the disease. He reported that zinnia approached a failure at Pullman and strawflowers near Seattle, Washington, during 1926. Strawflower near Los Angeles has often been a failure, and curly top appears to be the cause.

Surveys were made from 1925 to 1933 to determine the injury to ornamental flowering plants in the San Juan, Salinas, San Joaquin, and Sacramento valleys. Field investigations were conducted on the ranches of seed companies, the University Farm at Davis, and nurseries where cut flowers were sold. An examination was also made of flower gardens in parks, around schools, hotels, and homes, and flowering plants grown in cemeteries. A study of the symptoms of the disease on ornamental flowering plants was undertaken in naturally and experimentally infected plants.

METHODS OF TESTING PLANTS NATURALLY INFECTED WITH VIRUS DISEASES

Curly Top.—The methods of testing economic plants and weeds which were naturally infected with curly top have been described in a previous paper,[18] but a few changes in methods were made with ornamental flowering plants. A circular hole was cut in a piece of cardboard and the root system or branches of diseased plants were pulled through the hole and projected into a jar of water, while the foliage and flowers were enclosed in a cage. Sometimes branches were cut from a large number of diseased plants, and then groups of branches from different plants, 6 or 8 to a cage, were tested for curly top; or at other times entire plants were transplanted with the root system from the field and each was tested singly. Twenty previously noninfective male beet leafhoppers were fed for a period of 2 days on the diseased plants and then transferred to 2 healthy beet seedlings in cages; two were used in case one seedling died owing to damping-off. Males were used rather than females so as to avoid egg deposition. A high rate of mortality of the insects often occurred in the greenhouse because of unfavorable food plants. If symptoms of curly top failed to develop in from 1 to 2 weeks, the beets were examined daily in insect-proof chambers for a period of 3 months. If a beet developed curly-top symptoms, it was evident that the ornamental flowering plant had been naturally infected with the disease in the field.

Yellows.—It was sometimes difficult to determine whether an ornamental flowering plant was naturally infected with curly top or yellows, and hence tests were made for both diseases when symptoms could not be reliably distinguished. The two species of leafhoppers were placed on the diseased plant at the same time. Previously noninfective *Cicadula divisa* were exposed to the diseased plants for a period of 3 days and were then transferred to healthy aster and celery plants and the beet leafhoppers were transferred to 2 healthy beet seedlings. If the aster and celery plants developed symptoms of yellows or if the beets developed curly top, it was evident that the ornamental flowering plant had been infected with one of the diseases under natural conditions. No ornamental flowering plant has been shown to be naturally infected with the two diseases simultaneously up to the present time.

CURLY-TOP SYMPTOMS

Ornamental flowering plants sometimes showed reliable symptoms of curly top, and yet previously noninfective beet leafhoppers exposed to the naturally infected plants failed to transmit the virus to healthy sugar beets. A detailed description of the curly-top symptoms on the sugar beet has been given in a previous paper,[16] and only the reliable and constant foliage symptoms will be briefly described in this paper. The earliest symptom plainly visible to the eye is a cleared or transparent network of minute veins generally occurring on the innermost or youngest leaves of the beet. Another reliable and constant symptom of curly top, developing usually after the veinlets have become transparent, is the presence on the lower surface of the leaves of numerous protuberances or small elevations on the veins resembling tiny warts. As the disease progresses, nipple-like papillae and knot-like swellings resembling galls develop here and there on the distorted veins. A clear viscid liquid which later turns black often exudes from the petioles, midrib, or veins.

CHENOPODIACEAE, GOOSEFOOT OR SALTBUSH FAMILY

Common summer-cypress *(Kochia scoparia var. trichophila)* growing on the University campus at Davis was proved to be naturally infected with curly top. It was found that many of the plants were stunted and 40 per cent of the plants were dead owing to curly top.

The symptoms which developed on young plants experimentally infected with curly top were more pronounced than on naturally infected plants. The leaves at the apical end of the branches and secondary shoots were dwarfed and twisted (fig. 1 *A*). The leaves were often twisted into a spiral (fig. 1 *D*). The margin of the leaves and midrib was often sinuous
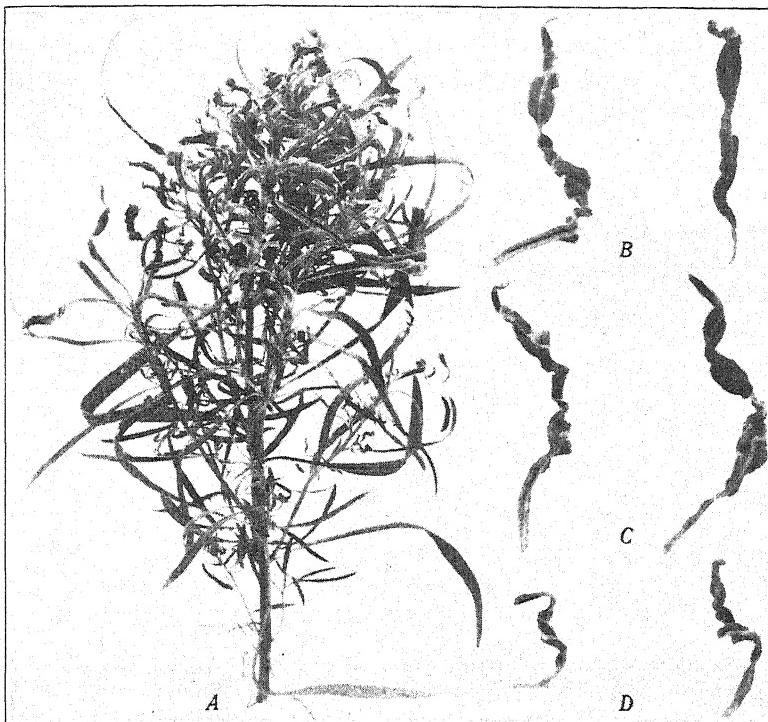
Fig. 1. Common summer-cypress (*Kochia scoparia* var. *trichophila*): *A*, Plant experimentally infected with curly top showing dwarfed and twisted leaves at the apical end of the branches and secondary shoots. *B*, *C*, Leaves with sinuous margins and midrib. *D*, Leaves showing spiral twist.

(fig. 1 *B*, *C*), with knot-like swellings on the midrib. The leaves showed blister-like elevations on the lower surface and cleared or transparent veinlets.

AMARANTHACEAE, AMARANTH FAMILY

Cockscomb *(Celosia argentea* var. *cristata)* grown in the flower gardens of the Merced County Hospital was demonstrated to be naturally infected with curly top. Some of the plants were stunted, but no detailed study of other symptoms were made.

NYCTAGINACEAE, FOUR-O'CLOCK FAMILY

Common four-o'clock *(Mirabilis jalapa)* growing on the Spreckels Ranch near King City in the Salinas Valley was proved to be naturally infected with curly top during the 1925 outbreak of the beet leafhopper. The leaves near the apices of the shoots showed a pronounced inward rolling toward the midrib and were thick, hard, and brittle.

Fig. 2. Grass pink *(Dianthus plumarius)*: apical end of branches of plants naturally infected with curly top showing secondary shoots with curled leaves.

Fig. 3. Grass pink *(Dianthus plumarius)*: left, apical end of branch of plant naturally infected with curly top showing proliferation of linear bracts around flower bud; right, branch from healthy plant showing flower bud surrounded by four linear bracts. (Keyes, San Joaquin Valley, November 9, 1932.)

CARYOPHYLLACEAE, PINK FAMILY

Grass pink *(Dianthus plumarius)* was demonstrated to be naturally infected with curly top in the San Joaquin Valley and showed pronounced symptoms of the disease. The internodes were shortened near the apices of the branches with secondary shoots (fig. 2) arising from the axils of the leaves. The larger leaves were curled (fig. 2) while the smaller leaves were dwarfed, curled, and twisted (fig. 4A) with cleared or transparent
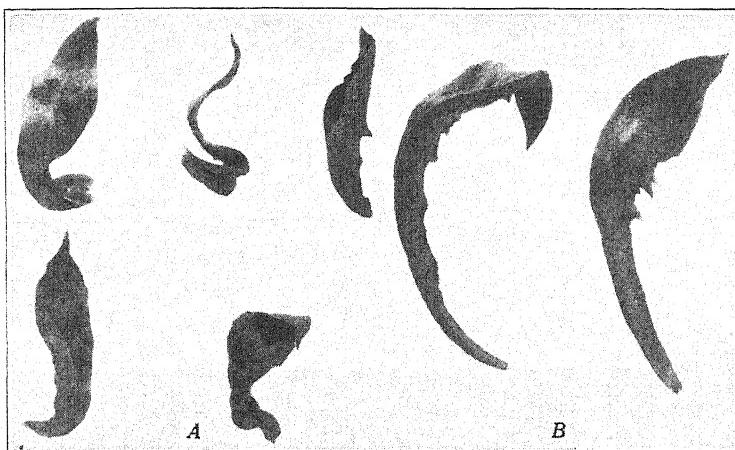

. Fig. 4. Grass pink *(Dianthus plumarius)*: *A*, Leaves from secondary shoots of plant naturally infected with curly top showing dwarfed, twisted, and puckered condition. *B*, Leaves from secondary shoots showing spines on the midrib. (Keyes, San Joaquin Valley, November 9, 1932.)

veinlets. On the lower surface of the leaves, protuberances were present on the distorted veins, resembling thorns on the midrib (fig. 4B). The upper surface of the smaller leaves was often puckered (fig. 4A). Numerous linear bracts were often present around the flower buds (plate 1A, fig. 3), instead of the normal number, four. The flowers on infected plants were dwarfed and white in color (plate 1A).

Carnation *(Dianthus caryophyllus)* appeared to be naturally infected with curly top in the San Joaquin Valley, but noninfective beet leafhoppers failed to recover the virus. The older leaves were often bent down, rolled, or twisted (fig. 5A). The leaves of the secondary shoots were dwarfed and sometimes formed a dense cluster (fig. 5C). A diseased plant sometimes showed proliferation of leaves on dwarfed axillary shoots and normal leaves on healthy shoots (plate 2A), indicating apparently a recovery from curly top. The flower buds at the apices of the branches were often dwarfed with the teeth of the calyx bent inward
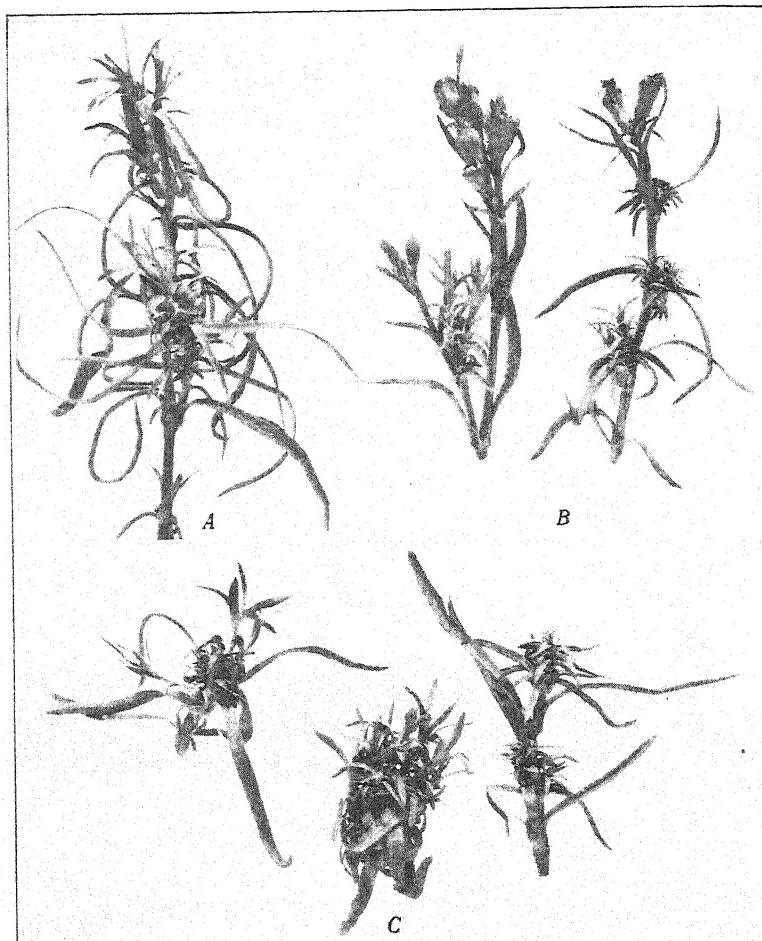
Fig. 5. Carnation (*Dianthus caryophyllus*) : *A*, Apical end of stem showing secondary shoots. *B*, Stems showing dried flower buds at the tip and rosettes of small leaves surrounding the flower buds at the end of the secondary shoots. *C*, Secondary shoots showing a dense cluster of dwarfed leaves. (Keyes, San Joaquin Valley, November 9, 1932.)

forming a bell-shaped bud (plate 1*B*) containing the withered flower parts. The flower buds at the tip of the secondary shoots were dwarfed with rosettes of small linear leaves (fig. 5*B*). The flowers sometimes were dwarfed or the petals were dry (plate 1*B*).

RANUNCULACEAE, CROWFOOT FAMILY

Turban and Persian buttercups *(Ranunculus asiaticus)* demonstrated to be naturally infected with aster yellows were collected by L. W. Massey in an acre field owned by Luther Gage, Carlsbad, California. This
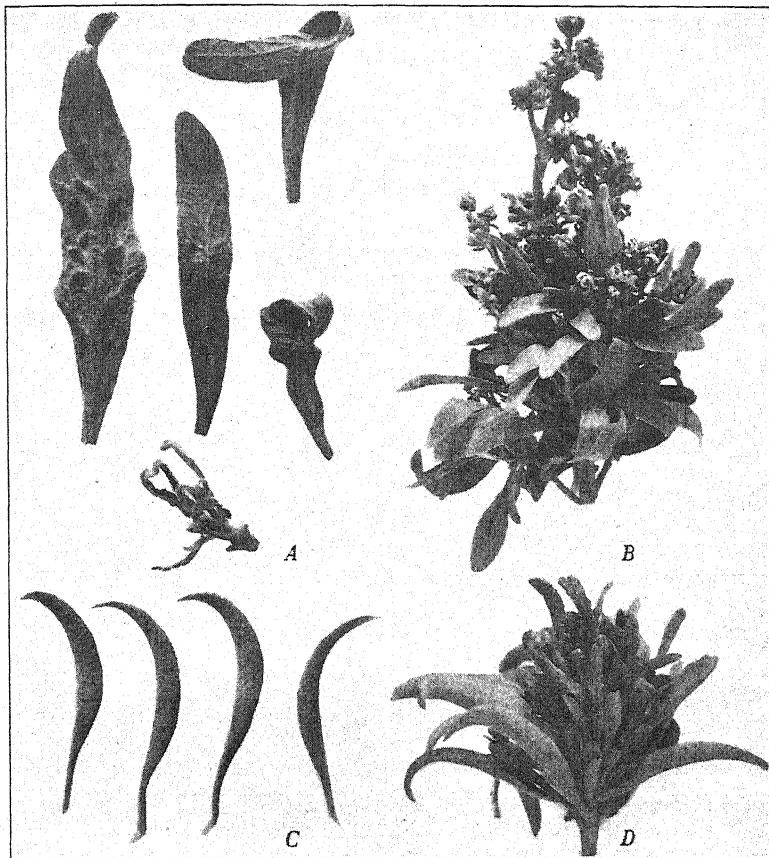
Fig. 6. Annual stock (*Mathiola incana* var. *annua*): *A*, Left, two leaves showing distorted veins with protuberances; right, two leaves showing curled and twisted blades; lower, secondary shoot with linear leaves (Davis, Sacramento Valley, November 1, 1932). *B*, Apical end of branch from plant naturally infected with curly top showing cluster of dwarfed flower stalks on secondary shoots (Chowchilla, San Joaquin Valley, November 9, 1932). *C*, Inward-curled leaves showing protuberances resembling tiny warts. *D*, Side view of terminal end of branch showing cluster of secondary shoots.

is the first record of the occurrence of aster yellows in San Diego County. The percentage of diseased plants was estimated at between 1 and 2 per cent. The diseased plants were yellow with numerous secondary shoots. The flowers were green and often dwarfed with petals reduced in size (plate 2*B*).

PAPAVERACEAE, POPPY FAMILY

California poppy *(Eschscholtzia californica)* growing on a ranch of a seed company near Salinas and in a flower bed near the Spreckels Agriculture Experiment Station was proved to be naturally infected with

yellows during the summer of 1929. The infected plants were dwarfed and chlorotic with many secondary shoots.

CRUCIFERAE, MUSTARD FAMILY

Stocks *(Mathiola incana, M. incana* var. *annua)*, grown in the Sacramento, San Joaquin, and Salinas valleys showed symptoms of curly top. The plants were stunted with shortened internodes with numerous axillary shoots bearing linear leaves at the apices of the branches (fig. 6A,
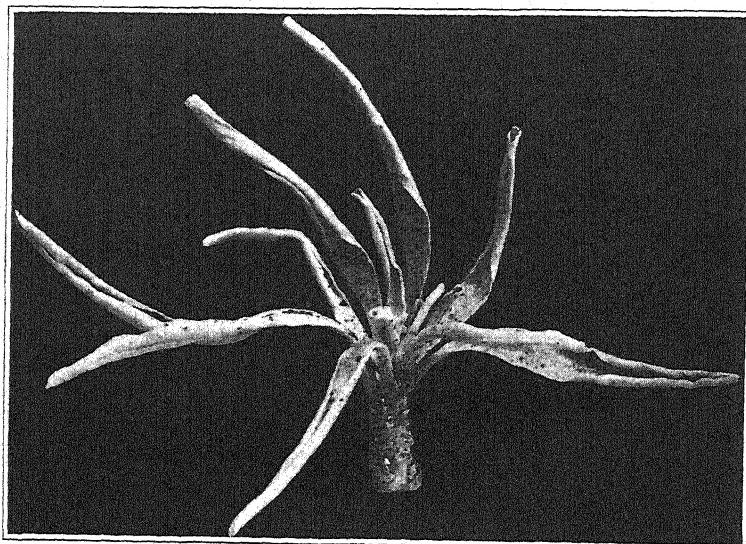

Fig. 7. Annual stock *(Mathiola incana* var. *annua)*: apical end of branch from a plant naturally infected with curly top showing white leaves with droplets of liquid which exuded from the blades, petioles, and stem. (Lodi, San Joaquin Valley, August 11, 1932.)

D). The leaves of the axillary shoots were sometimes twisted (fig. 6A) and often the margin of the leaves were curled or rolled inward. The veins on the lower surface of the leaves were distorted (fig. 6A) with protuberances resembling tiny warts (fig. 6C). The apices of the leaves of some plants were purple or yellow. The lower leaves of plants in an advanced stage of the disease were dry, and the leaves near the terminal end of the shoots were white. Brown droplets of liquid exuded from the blades, petioles, and stem (fig. 7), and were also present in experimentally infected stocks *(M. incana* var. *annua)*. The flower stalks from numerous axillary shoots formed a dense cluster (fig. 6B) and the flowers were often malformed with dried petals.

Enormous populations of beet leafhoppers were found on stocks in

the Sacramento and San Joaquin valleys, and many of the plants were stunted and often dead. Previously noninfective leafhoppers after feeding on the biennial or perennial stocks *(Mathiola incana)* removed from the field failed to transmit curly top to sugar beets, although the virus was recovered from experimentally infected annual stock *(M. incana var. annua)*. In one test 140 nymphs were captured on the biennial or perennial stocks grown on the University Farm at Davis and transferred in lots of 20 nymphs to 7 beet seedlings. One beet developed typical symptoms of curly top while 6 remained healthy. There is a possibility that some of the nymphs might have acquired the virus from weeds infected with curly top. In a previous paper[15] it was reported that different varieties of crucifers grown from seeds were inoculated with curly top by infective beet leafhoppers, but the transmission of the disease by previously noninfective leafhoppers after feeding on inoculated plants to sugar beets was not often accomplished.

Geraniaceae, Geranium Family

Fish geranium *(Pelargonium hortorum)* showed symptoms of curly top during the autumn in many localities in the San Joaquin Valley. Small elevations resembling tiny warts (plate 3*E*) were present on the lower surface of the older leaves but were absent on the younger leaves. In a cemetery near Los Banos where the geraniums were watered regularly, an inward rolling of the margin or cupping of some of the leaves occurred (plate 3*B*), but leaf rolling was not observed in other localities. The leaves sometimes were chlorotic (plate 3*E*), and entire plants were yellow and appeared to be in an advanced stage of the disease.

Fish geraniums which showed symptoms of curly top during the autumn were again examined during the following spring. The leaves on young shoots which grew from the roots showed reliable symptoms of the disease. The leaves were cupped inward, with sinuous veins (plate 3*D*), cleared veinlets (plate 3*C*), and protuberances on the lower surface. The older leaves were chlorotic between the veins, while the area in the vicinity of the veins was green. The apical leaves on the old branches showed protuberances on the lower surface, with dwarfed chlorotic leaves which developed from the nodes (plate 3*A*). The older branches were yellow instead of green. During the spring the virus was transferred by previously noninfective beet leafhoppers from 6 of 7 naturally infected plants to sugar beets, but during the autumn the virus was recovered from only 2 of the same 7 plants.

Cuttings from naturally infected geraniums collected during the autumn and kept in the greenhouse during the winter and spring failed to show symptoms of the disease on the younger leaves. These cuttings

Fig. 8. Dwarfed type of common nasturtium (*Tropaeolum majus*): *A*, Apical end of stems and secondary shoots showing dwarfed blades. *B*, Apical end of branch showing cupped leaves, secondary shoots with small blades and dried flowers. *C*, Flowers with enlarged pistils and cupped leaves. (Davis, Sacramento Valley, November 1, 1932.)

were exposed to infective beet leafhoppers and typical symptoms of curly top developed. The virus was recovered from the infected cuttings by previously noninfective leafhoppers and transferred to sugar beets.

Single giant-flowering hybrid geranium grown from seeds experimentally infected with curly top showed an inward rolling of the basal portion of the leaves or an inward or outward cupping of the leaves. The youngest leaves showed cleared or transparent veinlets. Previously noninfective beet leafhoppers transmitted the virus from the infected geraniums to sugar beets. A variety of geranium known as Mrs. Pollock was also experimentally infected with curly top.

TROPAEOLACEAE, TROPAEOLUM FAMILY

An unknown variety of nasturtium *(Tropaeolum majus)*, growing in the vicinity of the ranch houses of the California Packing Corporation near Tracy, was stunted (plate 4A) and most of the plants apparently had been destroyed by curly top. The virus was recovered from 1 of 3

Fig. 9. Dwarfed type of common nasturtium *(Tropaeolum majus)*: upper row left, normal flower from a healthy plant; second, dwarfed flower from a plant naturally infected with curly top; third and fourth, malformed flowers; lower row, flowers with dried sepals and petals. (Davis, Sacramento Valley, November 1, 1932.)

plants by previously noninfective beet leafhoppers and transferred to sugar beets.

It was found that about one-third of the common dwarf garden nasturtium *(Tropaeolum majus)* grown on the University Farm at Davis showed symptoms of curly top. Previously noninfective beet leafhoppers exposed to cuttings from 43 stunted plants failed to transmit curly top to sugar beets.

Nasturtiums apparently infected with curly top were also found in the interior districts of the Salinas Valley.

The outer or older leaves of naturally infected plants were usually yellow. Numerous secondary shoots (plate 4A) were present on naturally infected plants with dwarfed, cupped (fig. 8A, B) and sometimes puckered leaves with distorted veins. The dwarfed leaves were some-

times dry; occasionally the petiole below the blade was withered, while the remainder of the petiole was green. The leaves near the apices of the secondary shoots were dwarfed; they had small blades (fig. 8*A*) with the margins rolled inward.

The sepals and petals of immature flowers were withered or dry (fig. 9). The flower buds usually were dwarfed, chlorotic, and failed to expand. The pistil sometimes was greatly enlarged (fig. 8*C*).

Fig. 10. Pansy (*Viola tricolor hortensis*): apical end of branches from a plant naturally infected with curly top, showing dense clusters of secondary shoots.

Dwarfed mixed varieties of nasturtium were resistant to experimental infection with curly top. The virus was transferred to sugar beets by previously noninfective beet leafhoppers from only 2 of the 20 plants inoculated.

Carsner[2] reported that *Tropaeolum majus* was nonsusceptible to curly top.

VIOLACEAE, VIOLET FAMILY

Giant Trimardeau pansy *(Viola tricolor* var. *hortensis)* and Apricot Queen viola *(Viola cornuta)* were demonstrated to be naturally infected with curly top in the Salinas Valley.

The most conspicuous symptom of the disease was the dense cluster of chlorotic secondary shoots arising from the axil of the leaves near the tips of the branches (fig. 10) of the stunted plants. The margin of the leaves were rolled inward or cupped along the midrib, or the tips of the leaves were rolled toward the petioles. The youngest leaves showed cleared or transparent veinlets. The veins were wavy and bore small protuberances or papillae.

Dwarfed linear leaves surrounded the flower buds near the tip of the secondary shoots. The flower buds were often sessile, sometimes with a short peduncle. The flowers were dwarfed and frequently dry (fig. 11).

ONAGRACEAE, EVENING-PRIMROSE FAMILY

The Sybil Sherwood godetia *(Godetia grandiflora)* was found to be naturally infected with yellows in the Salinas Valley. Infected plants were chlorotic, with dwarfed curled leaves near the tips of the branches.

Fig. 11. Pansy *(Viola tricolor hortensis)* : upper row left, normal flower from healthy plant, and three dwarfed flowers from a diseased plant; lower row, dwarfed and dried flowers. (Greenfield, Salinas Valley, November 3, 1932.)

SOLANACEAE, NIGHTSHADE FAMILY

Common petunia *(Petunia hybrida)* was proved to be naturally infected with curly top in the Sacramento, San Joaquin, and Salinas valleys. The Rosy Morn variety *(Petunia hybrida)* grown in the flower beds adjacent to the University headhouses and greenhouses at Berkeley and the Double Ruffled variety *(Petunia hybrida)* grown along the highway near Davis were demonstrated to be naturally infected with curly top. Common petunias grown near Salinas, on the University Farm at Davis, and plants sent from Edgewood, Siskiyou County, were proved to be naturally infected with the disease.

Rosy Morn petunia transplanted in beds at Berkeley and then infected with curly top were stunted and developed numerous secondary shoots
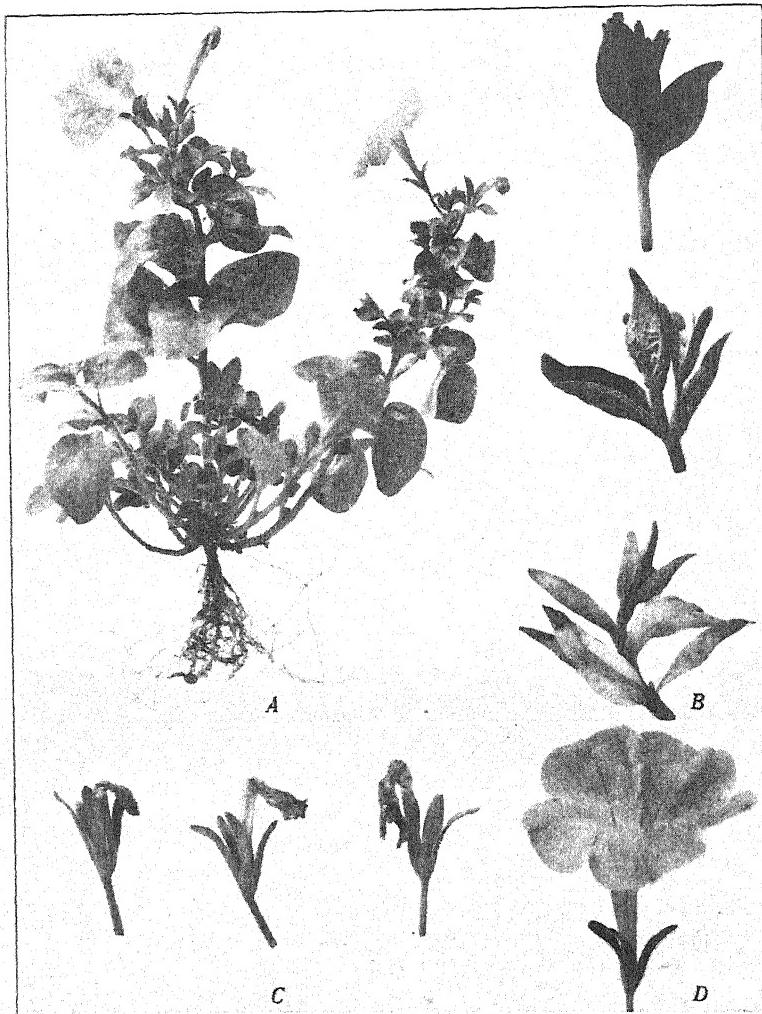
Fig. 12. Rosy Morn petunia (*Petunia hybrida*): *A*, Plant naturally infected with curly top showing abnormal development of secondary shoots. *B*, Upper, apical end of shoot from healthy check or control plant; center, apical end of shoot from plant naturally infected with curly top showing distorted veins with protuberances; apical end of shoot from plant in an advanced stage of the disease showing chlorotic leaves. *C*, Flowers from a plant naturally infected with curly top showing dried corollas. *D*, Flower from healthy check or control plant. (Berkeley, June 17, 1931.)

(fig. 12*A*) with dwarfed cupped leaves. Protuberances on the veins on the lower surface of the leaves of the secondary shoots and apical ends of the branches (fig. 12*B*) gave the veins a roughened appearance. The corolla of the flowers often failed to expand and became dry (fig. 12*C*). In the later stage of the disease the entire plant turned yellow and died.

Kunkel[8] has experimentally infected *Petunia hybrida* with aster yellows. Previously noninfective *Cicadula divisa* failed to transmit yellows to asters and celery from diseased petunias removed from the field.

DIPSACEAE, TEASEL FAMILY

Sweet scabiosa *(Scabiosa atropurpurea)* growing in a cemetery at King City in the Salinas Valley was proved to be naturally infected with curly top. The plants were stunted and chlorotic, but no other symptoms of the disease were noted.
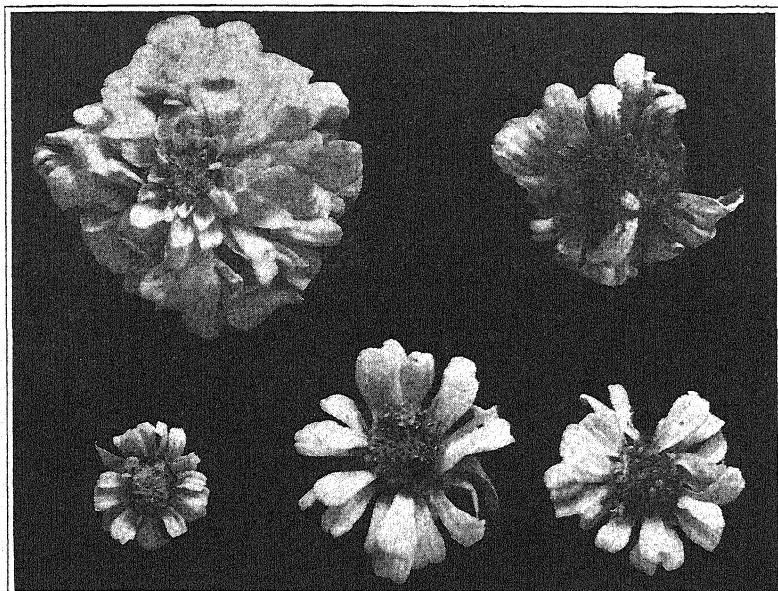
Fig. 13. Common zinnia *(Zinnia elegans)*: upper row left, normal flower from a healthy plant; remainder, dwarfed flowers with petals reduced in number, from a plant naturally infected with curly top. (Keyes, San Joaquin Valley, November 9, 1932.)

COMPOSITAE, COMPOSITE FAMILY

The Single Eldorado corn-marigold chrysanthemum *(Chrysanthemum segetum)* was demonstrated to be naturally infected with yellows in the Salinas Valley. The secondary shoots were chlorotic, the flower buds dwarfed, and petals reduced in number on the flowers.

Common zinnia *(Zinnia elegans)* grown near San Juan and Salinas was proved to be naturally infected with curly top. Diseased zinnias were more abundant when grown adjacent to garden, table, or red beets than when among ornamental flowering plants. The internodes near the apices of the branches were shortened, with chlorotic secondary shoots

(plate 2C) arising from the axils of the leaves. The flowers were dwarfed with the petals reduced in number (fig. 13).

In the greenhouse, *Zinnia elegans* and Mexican Double orange zinnia *(Zinnia haageana)* experimentally infected with curly top showed transparent or cleared veinlets on the youngest leaves (plate 2D), but this symptom could not be detected in the field.

Natural infection of zinnia with yellows disease was reported in a previous paper.[14] Three varieties of zinnias, commonly known as Double Giant Pink, Dahlia Flowered mixed, and Lilliput Scarlet Gem *(Zinnia elegans)*, grown near Salinas, were proved to be naturally infected with aster yellows. The virus was transferred by previously noninfective *Cicadula divisa* from the three varieties of zinnias to healthy celery. Naturally infected zinnias were often stunted and showed chlorotic secondary shoots. The symptoms of zinnia yellows, however, could not be distinguished from zinnia curly top on old plants; no study has been made in the field of the symptoms of the two diseases on young plants.

Common cosmos *(Cosmos bipinnatus)* was found to be naturally infected with curly top in the Salinas and San Joaquin valleys. The internodes were shortened toward the tips of the stems, and the apices of the branches and secondary shoots were yellow. The leaflets were often curled and twisted (fig. 14) and the petioles were bent downward; sometimes two adjacent petioles were bent parallel to the stem (fig. 14). The flower buds on the secondary shoots were dwarfed and surrounded by a cluster of small chlorotic leaves.

Crested cosmos *(Cosmos hybrida)* experimentally infected with curly top showed pronounced symptoms of the disease. The infected plants showed shortened internodes, chlorotic secondary shoots and apices of the branches, and curled and twisted leaves (fig. 15).

Calliopsis *(Coreopsis tinctoria)* growing in a cemetery near King City in the Salinas Valley was demonstrated to be naturally infected with curly top. The plants were stunted but showed no foliage symptoms of the disease.

Dwarfed French marigold *(Tagetes patula)* was proved to be infected with yellows on the University campus at Berkeley. The infected plants were stunted and chlorotic. A dense cluster of secondary shoots with dwarfed leaves and numerous flower buds developed at the tip of the branches (fig. 16A). The flower buds often failed to expand (fig. 16B). In the later stages of the disease, the plants died.

Aztec (African) marigold *(Tagetes erecta)* was demonstrated to be naturally infected with yellows in Berkeley. The infected plants were dwarfed and yellow and failed to blossom.[14]
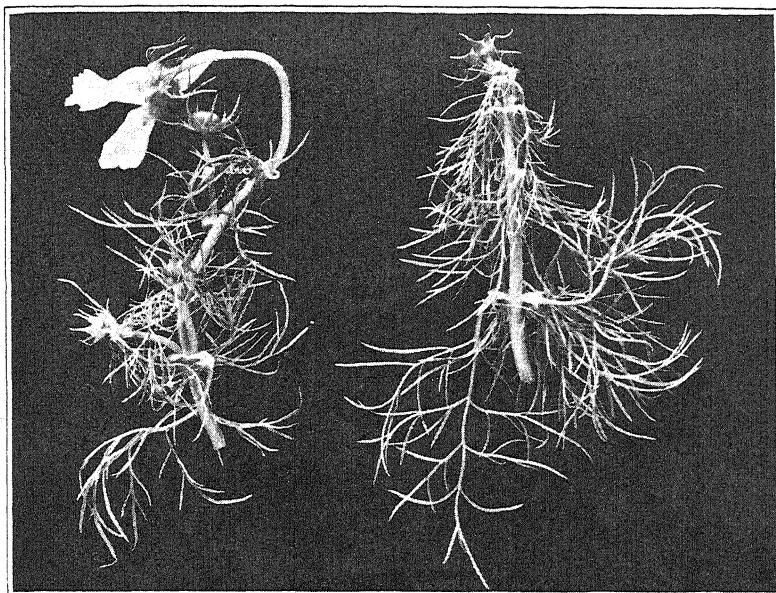
Fig. 14. Common cosmos (*Cosmos bipinnatus*) : apical ends of branches from a plant naturally infected with curly top showing bent petioles with curled and twisted leaves. (Salinas Valley, August 18, 1932.)

Fig. 15. Crested cosmos (*Cosmos hybrida*) : Left, apical end of shoot from healthy plant; right, apical shoot from a plant experimentally infected with curly top showing secondary shoots and curled and twisted leaves.

Strawflower *(Helichrysum bracteatum)* growing in a flower garden near Greenfield in the Salinas Valley, was demonstrated to be naturally infected with curly top. Numerous secondary shoots were present toward the tips of the branches (fig. 17A). The older leaves of the secondary shoots were curled outward with small protuberances on the distorted veins, while the younger leaves were linear and sometimes twisted into
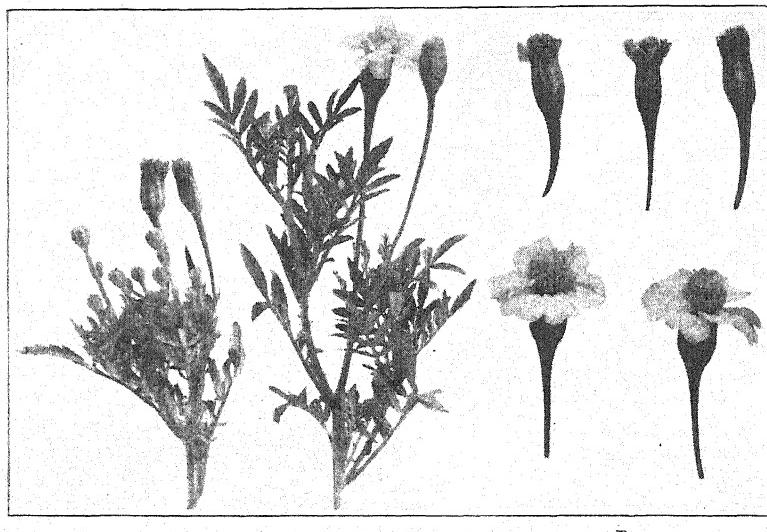
Fig. 16. Dwarfed French marigold *(Tagetes patula)*: *A*, Left, tip of branch from a plant naturally infected with aster yellows showing cluster of secondary shoots and numerous flower buds; right, shoot from a healthy plant. *B*, Upper row, flower buds which failed to blossom from a diseased plant; lower row, flowers from a healthy plant.

a spiral (fig. 17B). The flowers near the tips of the branches were dwarfed (plate 4B). Strawflower experimentally infected with curly top showed symptoms (fig. 17C) similar to those of naturally infected plants. The older leaves of the secondary shoots showed cleared or transparent veinlets.

Strawflower growing in the same flower garden near Greenfield was found to be naturally infected with yellows, as was demonstrated by the transfer of the virus from diseased plants by previously noninfective *Cicadula divisa* to aster and celery plants. Naturally infected plants were stunted and showed numerous chlorotic shoots. The flowers on the secondary shoots were green. Strawflower yellows could be distinguished from strawflower curly top by the absence of protuberances on the lower surface of the leaves.
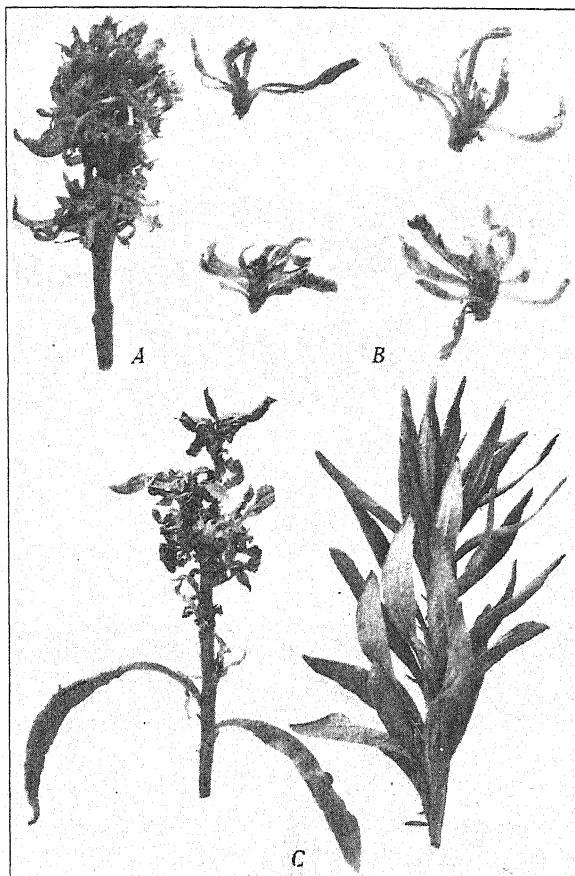
Fig. 17. Strawflower *(Helichrysum bracteatum)*: *A*, Apical end of branch from a plant naturally infected with curly top showing numerous secondary shoots bearing filamentous leaves. *B*, Secondary shoots showing twisted and spiral leaves (Greenfield, Salinas Valley, November 3, 1932). *C*, Left, apical end of branch from a plant experimentally infected with curly top showing secondary shoots; right, apical end of branch from a healthy check or control plant.

Summary

Fourteen species of ornamental flowering plants in 13 genera belonging to 10 families have been found to be naturally infected with curly top in California. Previously noninfective beet leafhoppers transferred the curly-top virus from the following 14 species of naturally infected plants to sugar beets.

Chenopodiaceae, goosefoot or saltbush family:
  Common summer-cypress (*Kochia scoparia* var. *trichophila*)
Amaranthaceae, amaranth family:
  Cockscomb (*Celosia argentea* var. *cristata*)
Nyctaginaceae, four-o'clock family:
  Common four-o'clock (*Mirabilis jalapa*)
Caryophyllaceae, pink family:
  Grass pink (*Dianthus plumarius*)
Geraniaceae, geranium family:
  Fish geranium (*Pelargonium hortorum*)
Tropaeolaceae, tropaeolum family:
  Garden nasturtium (*Tropaeolum majus*)
Violaceae, violet family:
  Giant Trimardeau pansy (*Viola tricolor* var. *hortensis*) Apricot Queen viola
    (*Viola cornuta*)
Solanaceae, nightshade family:
  Common, Rosy Morn, and Double Ruffled petunia (*Petunia hybrida*)
Dipsaceae, teasel family:
  Sweet scabiosa (*Scabiosa atropurpurea*)
Compositae, composite family:
  Common zinnia (*Zinnia elegans*), common cosmos (*Cosmos bipinnatus*),
    calliopsis (*Coreopsis tinctoria*), strawflower (*Helichrysum bracteatum*)

The curly-top virus was not recovered from the following 2 species of plants, but these plants showed reliable symptoms of the disease.

Caryophyllaceae, pink family:
  Carnation (*Dianthus caryophyllus*)
Cruciferae, mustard family:
  Stocks (*Mathiola incana, M. incana* var. *annua*)

Eight species and 3 varieties of ornamental flowering plants in 7 genera belonging to 4 families have been found to be naturally infected with aster yellows in California. Previously noninfective *Cicadula divisa* transferred the yellows virus from the following species and varieties of naturally infected plants to asters or celery:

Ranunculaceae, crowfoot family:
  Turban and Persian buttercups (*Ranunculus asiaticus*)
Papaveraceae, poppy family:
  California poppy (*Eschscholtzia californica*)
Onagraceae, evening-primrose family:
  The Sybil Sherwood godetia (*Godetia grandiflora*)
Compositae, composite family:
  The Single Eldorado corn-marigold chrysanthemum (*Chrysanthemum sege-
    tum*); common zinnia (*Zinnia elegans*): Double Giant Pink, Dahlia
    Flowered mixed, and Lilliput Scarlet Gem; Dwarfed French marigold
    (*Tagetes patula*); Aztec (African) marigold (*Tagetes erecta*); and
    strawflower (*Helichrysum bracteatum*)

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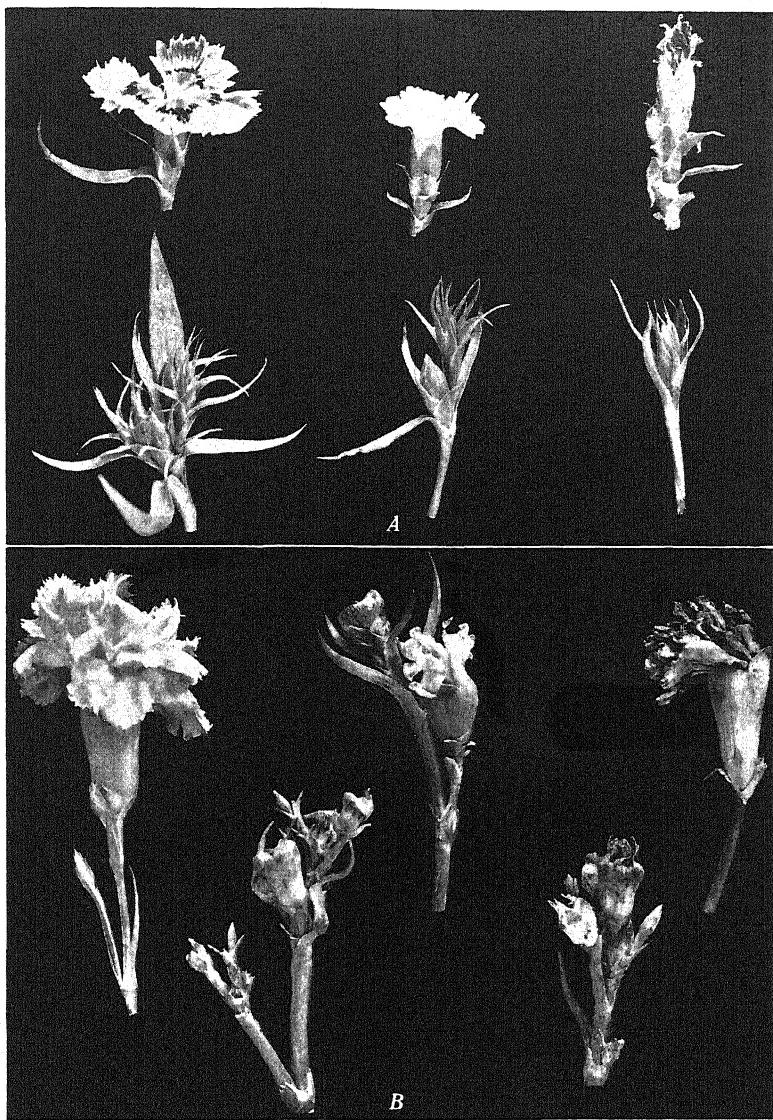
Plate 1. *A*, Grass pink (*Dianthus plumarius*): upper row left, normal flower from healthy plant; center, dwarfed flower, white in color instead of red or rose-colored; right, flower with dried petals, from plant naturally infected with curly top; lower row, flower buds with numerous bracts (Keyes, San Joaquin Valley, November 9, 1932). *B*, Carnation (*Dianthus caryophyllus*): upper row left, normal flower from healthy plant; center, dwarfed flower; right, flower with dried petals, from plant naturally infected with curly top; lower row, dried bell-shaped flower buds with no petals (Keyes, San Joaquin Valley, November 9, 1932).
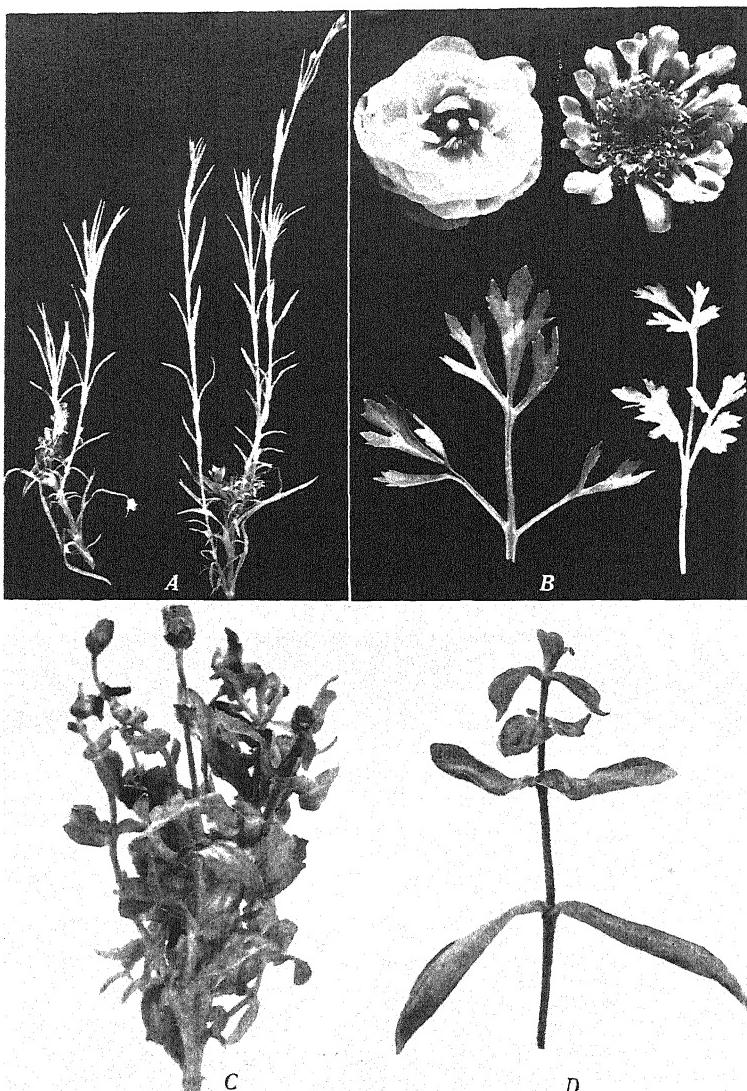
Plate 2. *A, Carnation (Dianthus caryophyllus)*: branches showing secondary shoots with clusters of dwarfed leaves, and apparently healthy stems with normal leaves from a plant naturally infected with curly top (Keyes, San Joaquin Valley, November 9, 1932). *B*, Turban and Persian buttercups *(Ranunculus asiaticus)*: upper left, normal flower from healthy plant; upper right, abnormal flower from a diseased plant showing petals reduced in size; lower left, normal leaf from healthy plant; lower right, chlorotic leaf from a diseased plant (Carlsbad, San Diego County, March 17, 1933, courtesy of L. W. Massey). *C*, Common zinnia *(Zinnia elegans)*: plant naturally infected with curly top showing secondary shoots (Fresno, San Joaquin Valley, November 9, 1932). *D*, Common zinnia (*Zinnia elegans)*: apical end of branch from a plant experimentally infected with curly top, showing cleared or transparent veinlets on the youngest leaves.
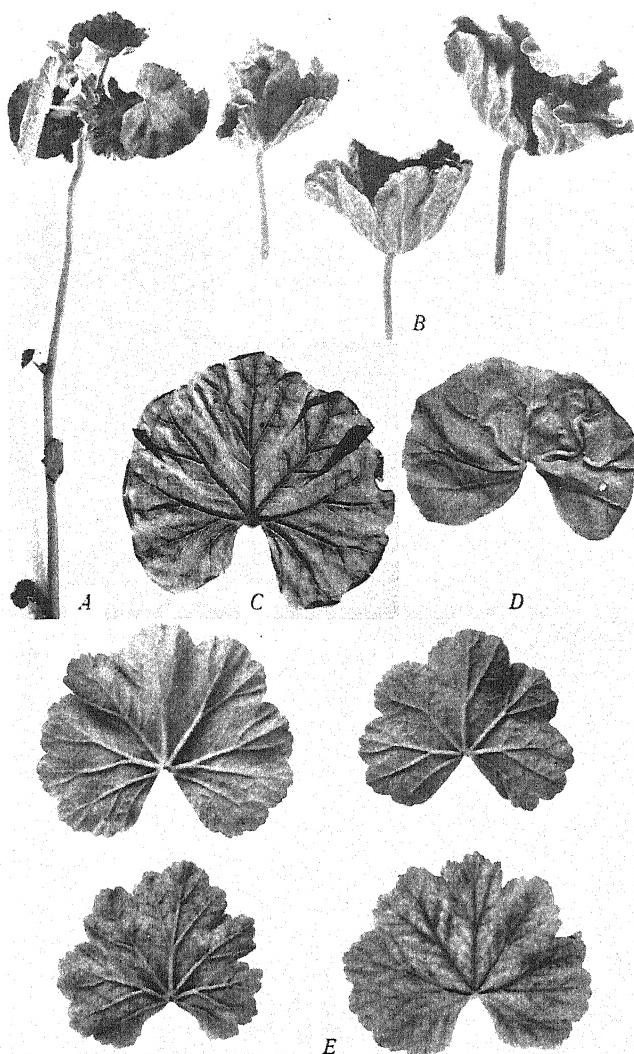
Plate 3. Fish geranium (*Pelargonium hortorum*): *A*, Cluster of apical leaves on old branch with dwarfed leaves at the nodes (Dos Palos, San Joaquin Valley, April 10, 1933). *B*, Leaves showing inward rolling of the margin or cupping of the blades (Los Banos, San Joaquin Valley, November 8, 1932). *C*, Leaf showing cleared or transparent veinlets (Dos Palos, San Joaquin Valley, April 10, 1933). *D*, Leaf showing sinuous veins (Dos Palos, San Joaquin Valley, April 10, 1933). *E*, Upper row, leaves from plant naturally infected with curly top, showing small elevations resembling tiny warts; lower row, leaves showing chlorotic areas between the veins (Dos Palos, San Joaquin Valley, November 8, 1933).
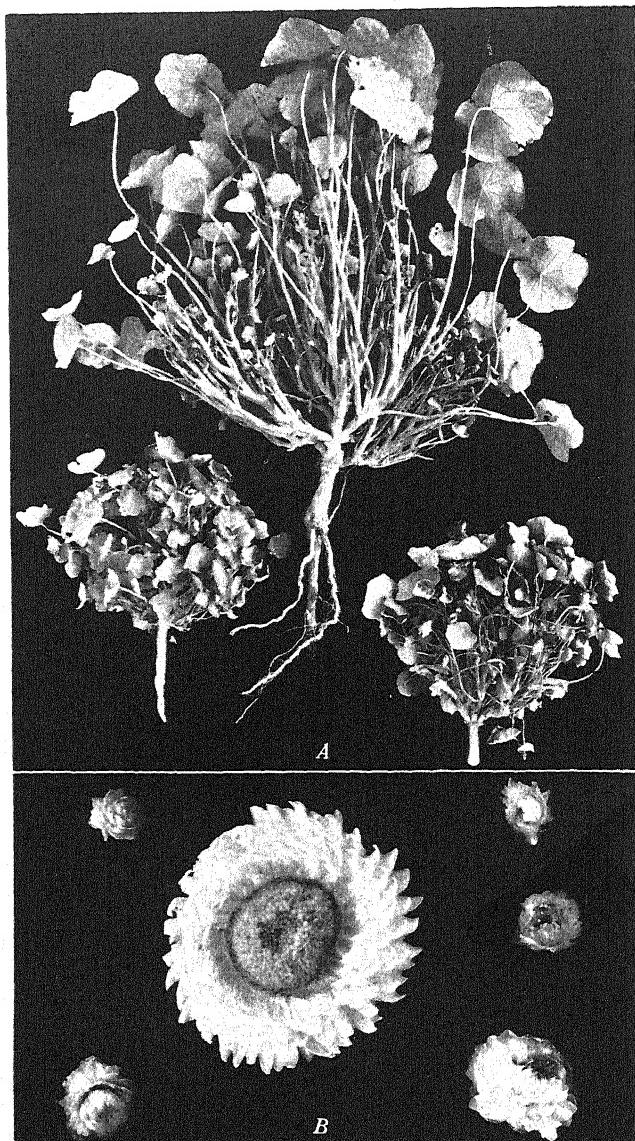
Plate 4. *A*, Dwarfed type of common nasturtium (*Tropaeolum majus*): two stunted and one large plant apparently naturally infected with curly top, showing numerous secondary shoots (Davis, Sacramento Valley, November 1, 1932). *B*, Strawflower (*Helichysum bracteatum*): center, normal flower from a healthy plant; grouped around it are five dwarfed flowers from a plant naturally infected with curly top (Greenfield, Salinas Valley, November 3, 1932).

WEED HOST RANGE AND OVERWINTERING OF CURLY-TOP VIRUS

HENRY H. P. SEVERIN

WEED HOST RANGE AND OVERWINTERING OF CURLY-TOP VIRUS[1]

HENRY H. P. SEVERIN[2]

(Contribution from the Division of Entomology and Parasitology, California Agricultural Experiment Station, coöperating with the United States Department of Agriculture, Bureau of Entomology.)

INTRODUCTION

IT IS IMPORTANT TO KNOW what plants growing in the cultivated areas and on the uncultivated plains and foothills are reservoirs of the curly-top virus. After sugar beets and other economic host plants of the beet leafhopper, *Eutettix tenellus* (Baker), are harvested, the adults of the overwintering generation feed on weeds and perennials during their flights from the cultivated areas to the uncultivated plains and foothills. After the pasture vegetation becomes green, the adults of the over-wintering generation leave the perennials and feed on the pasture vegetation. After the pasture vegetation becomes dry on the plains and foothills, the adults of the spring generation fly into the cultivated areas and are often abundant on favorable weeds. Some of these food plants of the beet leafhoppers serve as host plants of the curly-top virus.

A number of papers have appeared on the experimental infection of weeds with curly top, but the contributions on naturally infected weeds are limited. A review of the literature on this subject follows:

Boncquet and Stahl[1] experimentally infected dwarf mallow (*Malva rotundifolia* L.) with curly top and demonstrated that this weed growing in the vicinity of beets in the Salinas Valley was naturally infected with the disease.

Severin[5, 7] and Severin and Henderson[11] reported that 26 species of weeds and shrubs in 10 genera belonging to 6 families were experimentally infected with curly top, and 25 species of wild plants in 11 genera of 8 families were demonstrated to be naturally infected with curly top.

Carsner[2] found 11 species of wild plants and 3 species of economic plants belonging to 11 families susceptible to curly top and recovered the virus from 12 species. He reported 24 species of uncultivated and economic plants as nonsusceptible to curly top. In a later paper[3] he reported that the virus of curly top became attenuated when passed through certain weeds such as *Chenopodium murale* L., *Rumex crispus* L., and *Suaedea moquini* Greene.

TABLE 1

List of Plants Experimentally Infected with Curly Top

Family	Common name	Scientific name	Seasons duration	Source of plants
Urticaceae	Small nettle	*Urtica urens* L.	Annual	Seeds
Polygonaceae	Water smartweed	*Polygonum amphibium hartwrightii* Bissel	Perennial	Sacramento Valley
	Swamp smartweed	*Polygonum muhlenbergii* Wats.	Perennial	Sacramento Valley
	Common knotweed	*Polygonum lapathifolium* L.	Annual	San Joaquin Valley
	Lady's thumb	*Polygonum persicaria* L.	Annual	San Joaquin Valley
	Wire grass	*Polygonum aviculare* L.	Annual	San Joaquin Valley
	Curly dock	*Rumex crispus* L.[7]*	Perennial	Seeds
Chenopodiaceae	Arrowscale	*Atriplex phyllostegia* Wats.[11]	Annual	Seeds
	Bractscale	*Atriplex bracteosa* Wats.[11]	Annual	Seeds
	Brittlescale	*Atriplex parishii* Wats.[11]	Annual	Seeds
	Crownscale	*Atriplex coronata* Wats.[11]	Annual	Seeds
	Fogweed (silverscale)	*Atriplex argentea expansa* (Wats.)[11]	Annual	Seeds
	Garden orache (gardenscale)	*Atriplex argentea hillmanii* Jones	Annual	Sierra Nevada
		Atriplex hortensis rubra L.	Annual	Seeds, Idaho
	Heartscale	*Atriplex cordulata* Jepson[11]	Annual	Seeds
	Spear orache (spearscale)	*Atriplex patula hastata* L.[11]	Annual	Seeds
		Atriplex tularensis Coville[11]	Annual	Seeds
	Red orache (redscale)	*Atriplex rosea* L.[11]	Annual	Seeds
	Australian saltbush (fleshscale)	*Atriplex semibaccata* R.Br.[11]	Perennial	Seeds
	Ballscale	*Atriplex fruticulosa* Jepson[11]	Perennial	San Joaquin Valley, seeds
	Quailbrush (lenscale)	*Atriplex lentiformis* (Torr.) Wats.[11]	Perennial	San Joaquin Valley
		Chenopodium leptophyllum Wats.[11]	Annual	Seeds
	Lamb's quarters (white pigweed)	*Chenopodium album* L.[11]	Annual	Seeds
	Sowbane (nettleleaf goosefoot)	*Chenopodium murale* L.[11]	Annual	Seeds
	Mexican tea	*Chenopodium ambrosioides* L.[11]	Perennial	San Joaquin Valley
	Soap plant	*Chenopodium californicum* Wats.[11]	Perennial	Root
	Russian thistle	*Salsola kali tenuifolia* G. F. W. Mey.[11]	Annual	San Joaquin Valley
Amaranthaceae	Rough pigweed	*Amaranthus retroflexus* L.	Annual	San Joaquin Valley
	Tumbleweed	*Amaranthus graecizans* L.	Annual	San Joaquin Valley
		Amaranthus deflexus L.	Annual	San Joaquin Valley
Portulacaceae	Common purslane	*Portulaca oleracea* L.	Annual	Berkeley
Caryophyllaceae	Common chickweed	*Stellaria media* (L.) Cyr.	Annual	Berkeley

* Superscript number in parentheses refers to paper number in Literature Cited.

TABLE 1—(*Concluded*)

Family	Common name	Scientific name	Seasons duration	Source of plants
Cruciferae	Wild radish	*Raphanus sativus* L.	Annual	Seeds
	Charlock	*Brassica arvensis* (L.) B.S.P.	Annual	Seeds
	Shepherd's purse	*Capsella bursa-pastoris* (L.) Moench.[7]*	Annual	Seeds
Leguminosae	Bur clover	*Medicago hispida* Gaertn.[11]	Annual	Seeds
Oxalidaceae	Yellow sorrel	*Oxalis corniculata* L.	Perennial	Berkeley
		Oxalis corniculata atropurpurea Planch	Perennial	Berkeley
Geraniaceae		*Erodium botrys* Bertol.	Annual	San Joaquin Valley
	White-stem filaree	*Erodium moschatum* L'Her.[6]	Annual	Berkeley
	Red-stem filaree	*Erodium cicutarium* L'Her.	Annual	Seeds
Euphorbiaceae	Petty spurge	*Euphorbia peplus* L.	Annual	Berkeley
Malvaceae	Dwarf mallow	*Malva rotundifolia* L.[7]	Annual or biennial	Seeds
	Cheeseweed	*Malva parviflora* L.[7]	Annual or biennial	Seeds
Primulaceae	Poor man's weather-glass	*Anagallis arvensis* L.	Annual	Berkeley
Solanaceae	Tree tobacco	*Nicotiana glauca* Graham	Perennial	San Joaquin
		Solanum douglasii Dunal.[7]	Perennial	Seeds
		Physalis wrightii Gray	Annual	Seeds
	Stramonium	*Datura stramonium* L.[7]	Annual	Seeds
Plantaginaceae	Common plantain	*Plantago major* L.	Perennial	San Jose
		Plantago erecta Morris	Annual	San Joaquin Valley
Compositae	Common sow-thistle	*Sonchus oleraceus* L.	Annual	Berkeley
	Prickly sow-thistle	*Sonchus asper* L.	Annual	San Joaquin Valley
	Cotton-batting plant	*Gnaphalium chilense* Spreng.	Annual or biennial	Sacramento Valley
	Spiny clotbur	*Xanthium spinosum* L.	Annual	Seeds
	Common groundsel	*Senecio vulgaris* L.	Annual	Berkeley
	Mayweed	*Anthemis cotula* L.	Annual	Berkeley

* Superscript number in parentheses refers to paper number in Literature Cited.

According to Lackey[4] the attenuated curly-top virus was restored to its original virulence by passing it through common chickweed, *Stellaria media* (L.) Cyr. (plate 1 *A, B, C*).

Starrett[13] experimentally infected *Oxalis stricta* L. with curly top by means of infective beet leafhoppers and transferred the virus from the infected plants to sugar beets through the agency of previously noninfective beet leafhoppers.

In this paper new wild host plants of curly top are listed and also those previously recorded in the literature.[5, 7, 11] Field investigations

to determine the weeds which are naturally infected with curly top in the cultivated areas and on the uncultivated plains and foothills have been conducted over a period of sixteen years. Investigations to ascertain the weeds which could be experimentally infected with the disease were also made. Further investigations were conducted on the overwintering of the curly-top virus in host plants growing on the uncultivated plains and foothills, in perennial plants growing in the cultivated areas, and in the overwintering generation of beet leafhoppers.

METHODS

The methods used in determining whether weeds, economic plants, and ornamental flowering plants were naturally infected with curly top, have been described in previous papers.[5, 11, 12] The methods of experimentally infecting plants have also been described in a previous paper.[11]

PLANTS EXPERIMENTALLY INFECTED WITH CURLY TOP

A list of plants experimentally infected with curly top is given in table 1. These plants occur in the cultivated areas and were either grown from seeds or transplanted from the field.

As indicated in table 1, the weeds experimentally infected with curly top include 41 species of annuals, 3 species of annuals or biennials, and 13 species of perennials—a total of 57 species or varieties of weeds in 28 genera belonging to 16 families. The virus was recovered from each species of infected plant by previously noninfective beet leafhoppers and transferred to sugar beets.

PLANTS NATURALLY INFECTED WITH CURLY TOP

Table 2 lists the weeds and other wild plants which were demonstrated to be naturally infected with curly top. The list includes plants which were growing on the uncultivated plains and foothills and in the cultivated areas.

The plants growing on the uncultivated plains and foothills demonstrated to be naturally infected with curly top include, as shown in table 2, 11 species of annuals and 3 species of perennials—a total of 14 species in 13 genera belonging to 8 families.

In the cultivated areas 19 species of annuals, 3 species of annuals or biennials, and 4 species of perennials—a total of 26 species in 15 genera belonging to 9 families—were proved to be naturally infected with the disease.

Many plants in which the beet leafhopper has not been bred from eggs deposited under natural conditions up to the present time were experi-

TABLE 2

LIST OF PLANTS NATURALLY INFECTED WITH CURLY TOP

Family	Common name	Scientific name	Seasons duration	Valley in which plants were obtained
		Uncultivated plains and foothills		
Chenopodiaceae	Patata	*Monolepsis nuttalliana* (R. & S.) Wats	Annual	San Joaquin
	Ballscale	*Atriplex fruticulosa* Jepson[5]*	Perennial	San Joaquin
Cruciferae		*Thelypodium lasiophyllum* (H. & A.) Greene	Annual	San Joaquin
	Common pepper-grass	*Lepidium nitidum* Nutt	Annual	San Joaquin
	Shepherd's purse	*Capsella bursa-pastoris* (L.) Moench.	Annual	San Joaquin
Geraniaceae		*Erodium macrophyllum* H. & A.	Annual	San Joaquin
	Red-stem filaree	*Erodium cicutarium* L'Her.[5,6]	Annual	San Joaquin, Salinas
Leguminosae	Bur clover	*Medicago hispida* Gaertn.[5]	Annual	Bitterwater
		Lotus strigosus (Nutt.) Greene	Annual	San Joaquin
Malvaceae		*Modiola caroliniana* Don	Perennial	San Joaquin
Hydrophyllaceae		*Phacelia ramosissima* Dougl.	Perennial	San Joaquin
Plantaginaceae		*Plantago erecta* Morris	Annual	San Joaquin
Compositae		*Microseris douglasii* Gray	Annual	San Joaquin
		Baeria uliginosa (Nutt.) Gray	Annual	San Joaquin
		Cultivated areas		
Polygonaceae	Water smartweed	*Polygonum amphibium hartwrightii* Bissel[7]	Perennial	Sacramento
	Swamp smartweed	*Polygonum muhlenbergii* Wats.[7]	Perennial	Sacramento
	Common knotweed	*Polygonum lapathifolium* L.[7]	Annual	San Joaquin
	Lady's thumb	*Polygonum persicaria* L.[7]	Annual	San Joaquin
	Wire grass	*Polygonum aviculare* L.[7]	Annual	San Joaquin, Sacramento
Chenopodiaceae	Bractscale	*Atriplex bracteosa* Wats.[5,11]	Annual	San Joaquin
	Fogweed (silver-scale)	*Atriplex argentea expansa*(Wats.)[5,11]	Annual	San Joaquin
	Spear orache (spear-scale)	*Atriplex patula hastata* L.[11]	Annual	Sacramento
	Red orache (red-scale)	*Atriplex rosea* L.[5,11]	Annual	San Joaquin, Salinas
		Chenopodium leptophyllum Wats.[11]	Annual	San Joaquin
	Sowbane (nettleleaf goosefoot)	*Chenopodium murale* L.[11]	Annual	San Joaquin, Salinas
	Mexican tea	*Chenopodium ambrosioides* L.[11]	Perennial	San Joaquin
	Russian thistle	*Salso kali tenuifolia* G. F. W. Mey.[5,11]	Annual	San Joaquin, Salinas

* Superscript number in parentheses refers to paper number in Literature Cited.

TABLE 2—*(Concluded)*

Family	Common name	Scientific name	Seasons duration	Valley in which plants were obtained
		Cultivated areas—*(Continued)*		
Amaranthaceae	Rough pigweed	*Amaranthus retroflexus* L.[5]*	Annual	San Joaquin, Sacramento
	Tumbleweed	*Amaranthus graecizans* L.[5]	Annual	San Joaquin
		Amaranthus deflexus L.[5]	Annual	San Joaquin
Cruciferae	Charlock	*Brassica arvensis* (L.) B.S.P.[5,7]	Annual	Sacramento
	Shepherd's purse	*Capsella bursa-pastoris* (L.) Moench.[7]	Annual	Salinas
Leguminosae	Spanish clover	*Lotus americanus* (Nutt.) Bisch.	Annual	San Joaquin
Geraniaceae		*Erodium botrys* Bertol.	Annual	San Joaquin
Malvaceae	Dwarf mallow	*Malva rotundifolia* L.[7]	Annual or biennial	Salinas
	Cheeseweed	*Malva parviflora* L.[7]	Annual or biennial	San Joaquin, Sacramento
Solanaceae		*Solanum douglasii* Dunal.[5,7]	Perennial	San Joaquin, Salinas
		Physalis wrightii Gray[7]	Annual	San Joaquin
Compositae	Prickly sow-thistle	*Sonchus asper* L.	Annual	San Joaquin
	Cotton-batting plant	*Gnaphalium chilense* Spreng.	Annual or biennial	Sacramento
	Spiny clotbur	*Xanthium spinosum* L.	Annual	Salinas

* Superscript number in parentheses refers to paper number in Literature Cited.

mentally infected with curly top. The beet leafhopper was bred from eggs deposited under natural conditions from 8 species of plants growing on the uncultivated plains and foothills and from 38 species growing in the cultivated areas, as reported in a previous paper.[9]

SYMPTOMS OF CURLY TOP

The symptoms of curly top on the sugar beet, economic, and ornamental flowering plants have been described in four previous papers.[7, 8, 11, 12] A general description of the symptoms of the disease on weeds is given, with illustrations of a few typical examples, rather than a detailed description of the symptoms on each weed. Weeds infected with curly top show a variation in symptoms, but the following symptoms are characteristic of many diseased plants: stunting (figs. 1, 6A); abnormal development of secondary shoots (fig. 6C) with dwarfed leaves arising from the axils of the leaves (fig. 3); shortening of the internodes; dwarf-
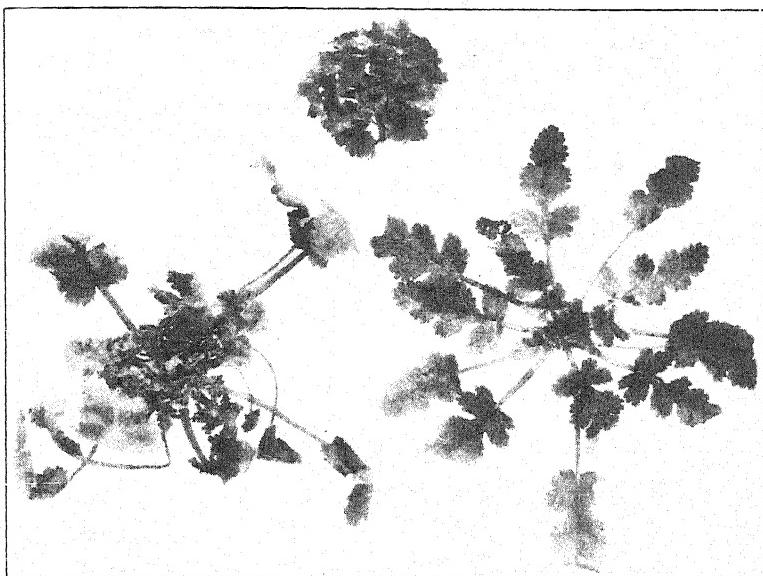
Fig. 1. *Erodium botrys:* left and upper, stunted plants naturally infected with curly top showing curled leaves; right, healthy plant. Niles Garden, March 6, 1934.
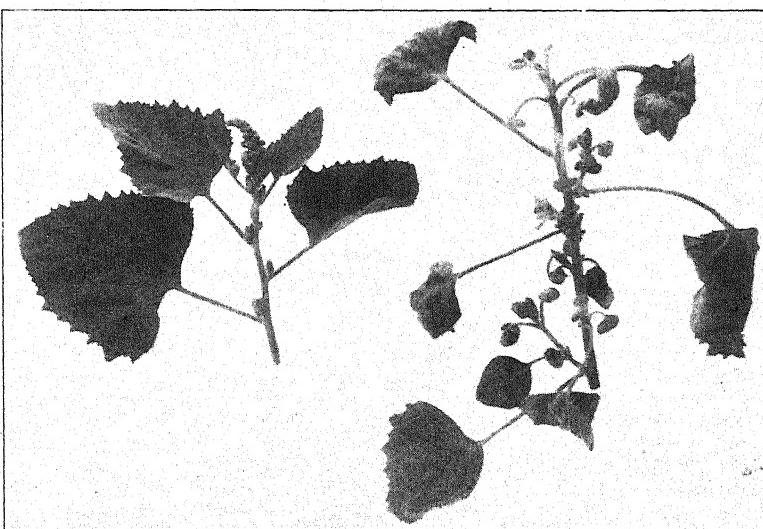
Fig. 2. Garden orache or gardenscale (*Atriplex hortensis rubra*): left, shoot from check or control plant on which noninfective beet leafhoppers had fed; right, shoot from a plant experimentally infected with curly top showing secondary shoots arising from the axil of the leaves bearing dwarfed, balled leaves.

Fig. 3. Rough pigweed (*Amaranthus retroflexus*) experimentally infected
with curly top showing outward-cupped leaves and axillary shoots.
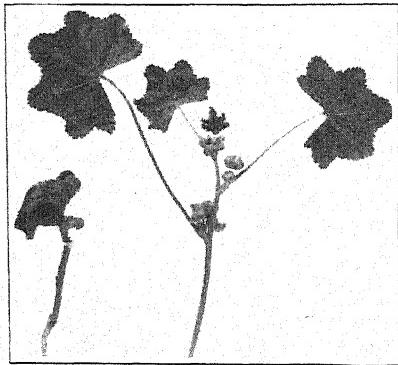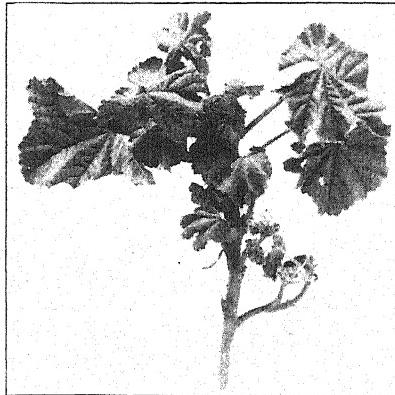
Fig. 4   Fig. 5

Fig. 4. Cheeseweed (*Malva parviflora*): left, shoot from a plant experiment-
ally infected with curly top showing outward-cupped leaves; right, shoot from a
check or control plant on which noninfective leafhoppers had fed.

Fig. 5. Cheeseweed (*Malva parviflora*) naturally infected with curly top show-
ing stunted plants with outward-cupped leaves.

ing, curling (fig. 1; plate 1*B*), cupping (figs. 3, 4, 5), rolling (plate 1 *A*,
E), balling (fig. 2), twisting (fig. 6*A*), puckering, mottling, and chlorosis
of the leaves; cleared or transparent veinlets (plate 2*A*); vein distortions
(fig. 6, *B*, *D*); and protuberances (plate 1*D*; plate 2*B*) on the lower
surface of the leaves.
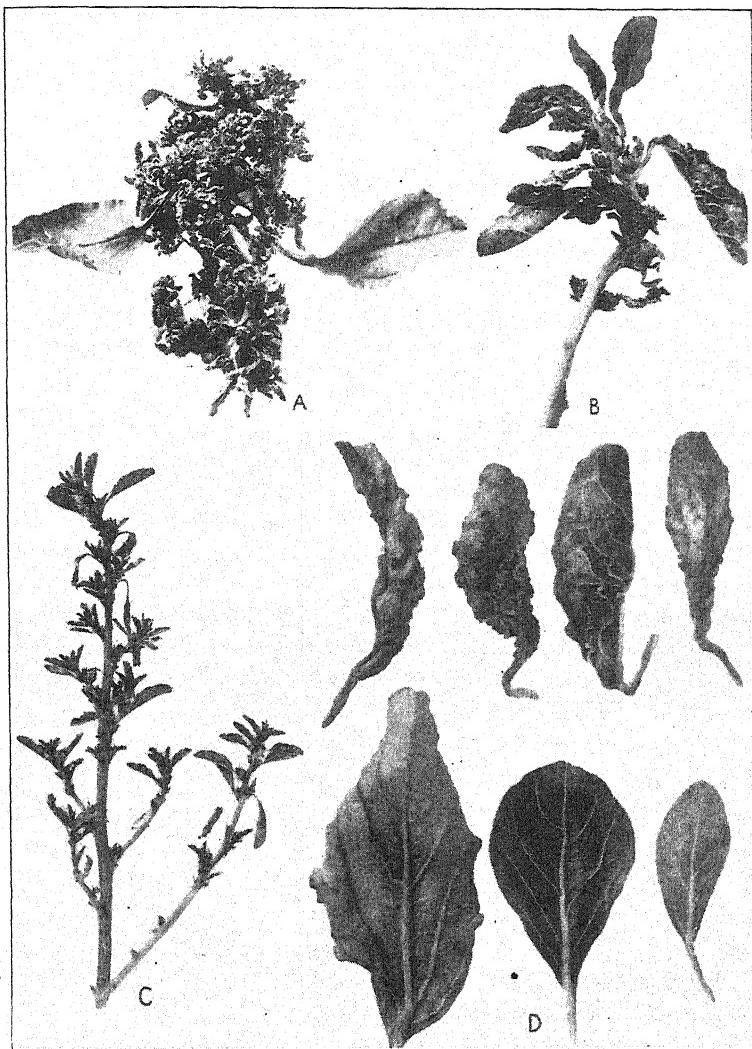
Fig. 6. Tumbleweed (*Amaranthus graecizans*): *A*, Plant naturally infected with curly top showing stunting and compact mass of twisted leaves. *B*, Branch showing leaves with vein distortions. *C*, Branch from a plant experimentally infected with curly top showing secondary shoots arising from the axil of the leaves. *D*, upper row, leaves showing vein distortions; lower row, left, leaf showing protuberances on the lower surface; center and right, leaves from the same plant showing no vein distortions and protuberances.

OVERWINTERING OF CURLY-TOP VIRUS

In Host Plants on Uncultivated Plains and Foothills.—The annual host
plants in which the curly-top virus overwinters on the uncultivated
plains and foothills are listed in table 2. The most important of these,
owing to their wide distribution, are as follows: common pepper-grass
(*Lepidium nitidum*), red-stem filaree (*Erodium cicutarium*), and
Plantago erecta. There are probably other important annuals in which
the curly-top virus overwinters on the uncultivated plains and foothills.
After the rains germinate the seeds of the pasture vegetation, small
annual plants infected with curly top succumb rapidly after symptoms
of the disease develop, but older deep-rooted plants infected with the
disease survive longer, and are therefore a factor in the overwintering
of the virus.

A test was made to determine the percentage of red-stem-filaree plants
which were naturally infected with curly top. All of the plants were
collected on March 2, 1926, in an area covering about 2 square yards on
a foothill bounding the entrance of Big Panoche Pass, where a large
congregation of overwintering leafhoppers occurred. Fifty red-stem-
filaree plants were selected; many small plants were dry and were re-
jected. Previously noninfective beet leafhoppers, 5 to a plant, were fed
for a period of 3 days on these 50 red-stem filaree plants and were then
transferred to 50 healthy sugar-beet seedlings. Five sugar beets devel-
oped typical curly-top symptoms and 45 beets remained healthy. The
5 red-stem filaree plants from which curly top was transmitted to sugar
beets showed reddish outer leaves and malformed inner leaves (figs.
7, 8).

In an earlier test previously reported,[6] 50 red-stem filaree plants
were collected on April 8, 1924, in depressions of squirrel mounds in
Little Panoche Pass and only 1 plant was demonstrated to be naturally
infected with the disease.

Among the plants belonging to the family Chenopodiaceae, to which
the sugar beet belongs, a single perennial species growing on the uncul-
tivated plains and foothills has been demonstrated to be naturally in-
fected with curly top; ballscale (*Atriplex fruticulosa*), a small spread-
ing saltbush often grazed down by cattle and sheep to a mat-like form,
and commonly found on the plains and foothills of the San Joaquin
Valley, has been proved to be naturally infected with the disease. Four
plants transplanted from the uncultivated plains were experimentally
infected with curly top, and the virus was repeatedly recovered by pre-
viously noninfective beet leafhoppers and transferred to beets during

Fig. 7. Red-stem filaree (*Erodium cicutarium*) naturally infected with curly top showing twisted petioles and inward-curled leaflets.

Fig. 8. Red-stem filaree (*Erodium cicutarium*) naturally infected with curly top with outer leaves removed, showing curled petioles and leaves; lower right, drooping flower.

a period of six months from May to October inclusive, when the tests were discontinued.

Two other perennial species growing on the plains and foothills in the San Joaquin Valley were demonstrated to be naturally infected with curly top as follows: (1) *Modiola caroliniana* belonging to the family Malvaceae, introduced from the southeastern part of the United States into California; and (2) *Phaceolia ramosissima*, belonging to the family Hydrophyllaceae, and occurring on the foothills and mountain slopes at an elevation of 200 to 9,000 feet almost throughout California, north to Washington, and south to Lower California.

In Host Plants in Cultivated Areas.—Four perennials growing in the cultivated areas and demonstrated to be naturally infected with curly top are listed in table 2. Since the annuals become dry during the autumn, they play no role in the overwintering of the virus.

Repeated tests were made to recover the curly-top virus from Mexican tea (*Chenopodium ambrosioides*), a perennial weed demonstrated to be naturally infected with the disease in the San Joaquin Valley. The virus was transferred from 4 naturally infected plants by different lots of previously noninfective beet leafhoppers to sugar beets during a period of 1 year, after which no further tests were made.[11]

Among economic plants, Hairy Peruvian alfalfa (*Medicago sativa*) and horse-radish (*Amoracia rusticana*), both perennials; Single or Plain parsley (*Petroselinum hortense*), a biennial or short-lived perennial; and potato (*Solanum tuberosum*), a herbaceous plant, were demonstrated to be naturally infected with curly top.[7, 11] The virus was rarely recovered from naturally infected horse-radish plants during the summer, and not at all during the autumn. The virus was not recovered from cuttings grown from naturally infected horse-radish roots.[7] No experiments have been conducted up to the present time to prove potato-tuber transmission of the disease, nor to test whether the virus could be recovered during the autumn and winter from alfalfa and parsley.

Among the ornamental flowering plants, grass pink (*Dianthus plumarius*) and fish geranium (*Pelargonium hortorum*), both perennials; common four-o'clock (*Mirabilis jalapa*) and common garden petunia (*Petunia hybrida*), both perennials grown as annuals; and pansy (*Viola tricolor hortensis*), an annual or short-lived perennial, were proved to be naturally infected with curly top.[12] Carnation (*Dianthus caryophyllus*), a perennial, and stocks (*Mathiola incana*), a biennial or perennial, showed typical symptoms of curly top under field conditions, but noninfective leafhoppers failed to transmit the virus from these plants to sugar beets.

In Beet Leafhopper.—The curly-top virus rarely overwinters in the male beet leafhopper, since most of the males die during the winter.[10] The average period of infectivity during the adult life of 10 females which completed the nymphal stages on diseased beets was 83.9 days, followed by an average period of 50.1 days between the last infection and death of the insect. When the females lived for a long time, the infective power was lost in most cases. The infective power is not retained during the adult life of the overwintering female beet leafhoppers unless they reinfect themselves during the winter; but in that case the virus does overwinter in the females.

SUMMARY

The weeds growing on the uncultivated plains and foothills and in the cultivated areas experimentally infected with curly top include 57 species in 28 genera belonging to 16 families.

The wild plants growing on the uncultivated plains and foothills demonstrated to be naturally infected with curly top include 14 species in 13 genera belonging to 8 families.

In the cultivated areas 26 species of weeds in 15 genera belonging to 9 families were found to be naturally infected with the disease in nature.

The curly-top virus overwinters in 11 species of annuals and 3 species of perennial wild plants growing on the uncultivated plains and foothills. Previously noninfective beet leafhoppers repeatedly recovered the virus from a perennial—ballscale (*Atriplex fruticulosa*)—during a period of six months, when the tests were discontinued.

Four species of perennials and 3 species of weeds sometimes annual and sometimes perennial growing in the cultivated areas were demonstrated to be naturally infected with curly top. The virus was repeatedly recovered from a naturally infected perennial—Mexican tea (*Chenopodium ambrosioides*)—during a period of one year, after which no further tests were made. The virus does not overwinter in the annuals growing in the cultivated areas, since these become dry during the autumn.

The following economic plants which may enable the virus to overwinter were demonstrated to be naturally infected with curly top: Hairy Peruvian alfalfa (*Medicago sativa*) and horse-radish (*Amoracia rusticana*) both perennials; Single or Plain parsley (*Petroselinum hortense*), a biennial or short-lived perennial; and potato (*Solanum tuberosum*), a herbaceous plant. The virus was rarely recovered from naturally infected horse-radish during the summer, and not at all dur-

ing the autumn, nor from cuttings grown from naturally infected horse-radish roots.

The following ornamental flowering plants were demonstrated to be infected with curly top under natural conditions : grass pink (*Dianthus plumarius*) and fish geranium (*Pelargonium hortorum*), both perennials; common four-o'clock (*Mirabilis jalapa*) and common garden petunia (*Petunia hybrida*), both perennials grown as annuals; and pansy (*Viola tricolor hortensis*), an annual or short-lived perennial. The virus was not recovered from carnation (*Dianthus caryophyllus*), a perennial, and stocks (*Mathiola incana*), a biennial or perennial, although these showed typical symptoms of curly top under field conditions.

The curly-top virus rarely overwinters in the male beet leafhopper, since most of the males die during the winter. The infective power is not retained during the adult life of the female beet leafhoppers unless they reinfect themselves during the winter, but in that case the virus overwinters in the females.

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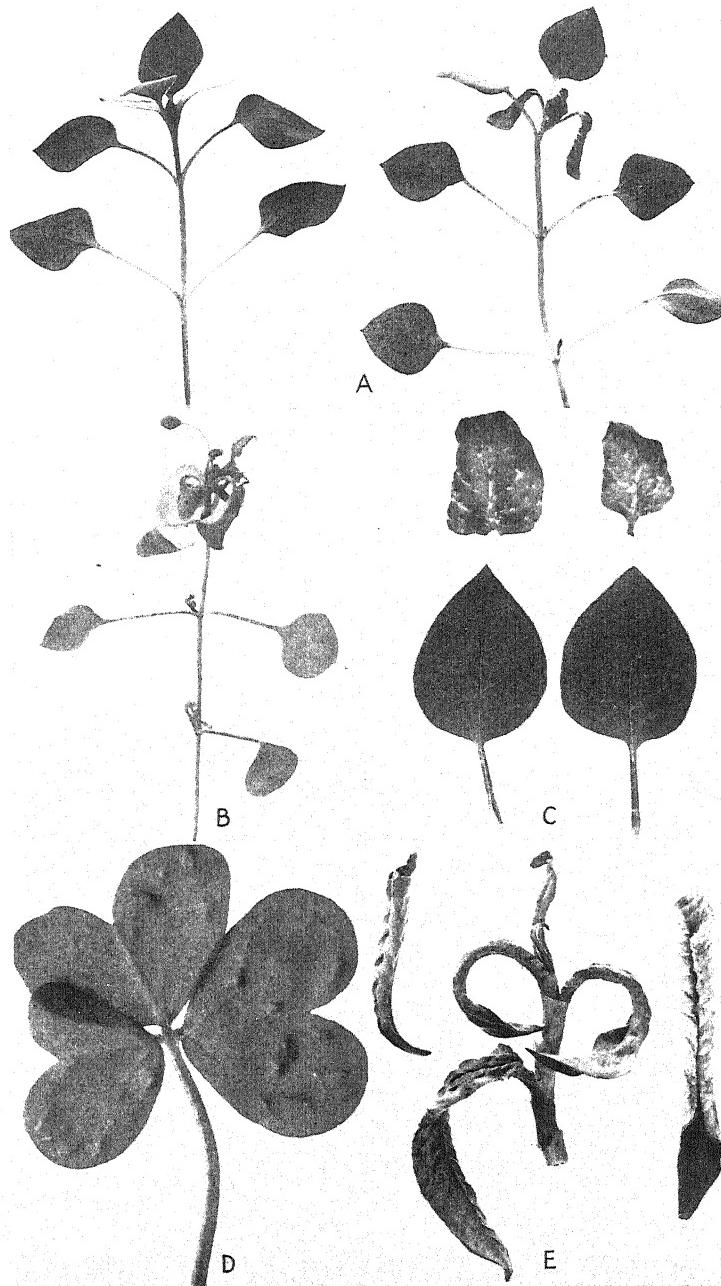
Plate 1. *A*, Common chickweed (*Stellaria media*): left, healthy plant; right, plant experimentally infected with curly top showing rolled leaves. *B*, Plant in an advanced stage of the disease showing curled apical leaves. *C*, Upper leaves from an infected plant showing protuberances; lower leaves from a healthy plant. *D*, Yellow sorrel (*Oxalis corniculata*): leaf from a plant experimentally infected with curly top showing protuberances. *E*, Swamp smartweed (*Polygonium muhlenbergii*) naturally infected with curly top, showing inward-rolled leaves.

A
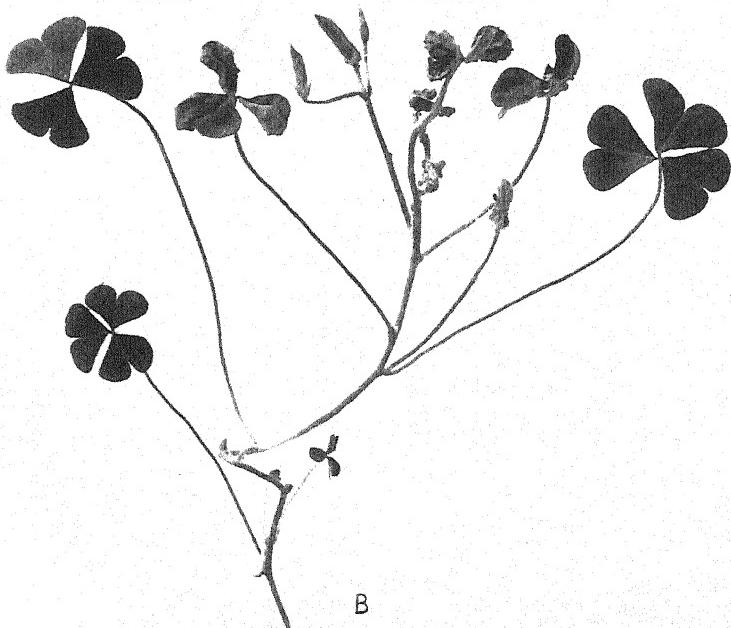
B

Plate 2. *A*, Prickly sow-thistle (*Sonchus asper*): left, leaf from a healthy plant; center and right, leaves from a plant experimentally infected with curly top showing cleared or transparent veinlets. *B*, Yellow sorrel (*Oxalis corniculata*) experimentally infected with curly top showing protuberances on the curled leaves.

VITAMIN-A DEFICIENCY IN TURKEYS[1]

W. R. HINSHAW[2] AND W. E. LLOYD[3]

LOSSES FROM OBSCURE CAUSES on certain California turkey ranges, where green feed is limited during much of the growing season, suggested the need for studies on vitamin-A deficiency in turkeys. The experiments reported herein were outlined to determine the possible relation of A-avitaminosis[4] to turkey mortality and to obtain information concerning the effect on turkeys of various vitamin-A levels.

A-avitaminosis in chickens has been fully described by Beach[1], Emmett and Peacock[3], Seifried[10], Elvehjem and Neu[4], and many other investigators, so that the pathological changes in chickens are well established. Chicks were included in one experiment as a control on the methods used and as a basis for comparing A-avitaminosis in the two species.

Scott and Hughes[9], using yellow corn as the source of vitamin A, showed that turkeys required more vitamin A than did chickens.

This paper is concerned chiefly with the disease phases of the problem. Because published data on the vitamin-A requirements of turkeys are limited, the results on the comparative value of dehydrated alfalfa-leaf meal for turkeys and chickens are also included. Certain vitamin-A liver storage data collected in connection with the experiments have already been published by Guilbert and Hinshaw.[6]

EXPERIMENTAL METHODS

Day-old poults and chicks were used for all the experimental work. The poults were hatched on June 21 and July 5, 1932, from eggs laid by pedigreed Bronze turkey hens from the Experiment Station breeding flock; the chicks on June 29 from eggs of the Station Single Comb White Leghorns. The parent stock of both the poults and the chicks received essentially the same rations, containing adequate vitamin A in the form of yellow corn, dehydrated alfalfa-leaf meal,[5] and freshly cut greens.

The basal ration used throughout was as follows: 25 pounds ground white corn, 25 pounds ground barley, 25 pounds ground wheat, 10 pounds fish scrap, 10 pounds dried milk, 3 pounds bone meal, 2 pounds ground limestone, 0.5 pound salt. Before grinding, the white corn was hand-picked to remove all yellow kernels, amounting to about 1 per cent.

This basal ration was supplemented with alfalfa meal and with finely chopped fresh alfalfa, to supply the various levels of vitamin A. According to feeding tests on rats, the alfalfa meal contained at least 230 Sherman rat units per gram; according to chemical analysis, 130 gamma of carotene per gram. The alfalfa-leaf meal was used as the source of vitamin A because of its availability to the turkey grower, because of its high vitamin-A content, because of the ease with which it could be mixed with the ration, and because of the need for information on the amounts of alfalfa meal required for turkey rations.

In the first experiment, the day-old poults were divided into 6 groups of 42 each. Lot 6 received the basal ration; lot 1 the basal ration plus freshly chopped green alfalfa; and lots 2, 3, 4, and 5 the basal ration plus 8, 4, 2, and 1 per cent of alfalfa meal, respectively. When hatched, the chicks were divided into 6 groups of 42 each and were placed in the pens with the week-old poults. The chicks and poults were reared together throughout the experiment.

In the second experiment the poults were divided into 5 groups of 29 each and were treated identically with those in the first experiment except that the group (lot 5) receiving 1 per cent alfalfa meal was omitted.

The birds were brooded in a long house divided into pens, with outdoor cement sun-porches, where from all indications they obtained a plentiful supply of the antirachitic factor. After being kept in the original pens until 12 weeks old, they were moved to an open-front house, with cement runs. The chicken cockerels were removed from the pens after 16 weeks to prevent fighting and injury to the turkeys.

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[5] For the sake of brevity, dehydrated alfalfa-leaf meal may hereafter be referred to in the text as alfalfa meal.

Walnut and fig trees shaded portions of the cement runs, but in most cases could be fenced off so that no leaves fell in the yards. The groups getting green feed were placed in the pens most exposed to falling leaves, and every effort was made to keep the yards free of leaves. For the last 3 months of the experiment, when the trees were shedding more rapidly, the birds were kept indoors.

The birds were started on sand as litter, but rice hulls were substituted after 2 weeks. Since a little water-grass *(Echinochloa crus-galli)* seed was present in the rice hulls, each lot got a limited quantity each time the litter was changed; but, as all pens were treated alike, this procedure presumably did not affect the final results.

Every chick and poult that died was subjected to a careful postmortem examination for fungal, bacterial, and protozoan infections, and for indications of vitamin-A deficiency.

When each experiment was closed, at least 4 birds from each lot were killed for autopsy. As a further check, their livers were tested for vitamin A by the antimony trichloride method. Three turkeys from each lot and three chickens each from lots 4 and 5 were placed in a new group and fed on the basal ration, to determine biologically the amount of vitamin-A storage. The remainder were placed together on a normal ration and later, when fully recovered, were sold for market.

EFFECT OF VITAMIN-A-FREE DIETS

Because the disease manifestations in turkeys receiving a completely deficient vitamin-A ration have not been previously described, they are presented below in some detail. Later they will be compared with observations on the disease in turkeys receiving different levels, but inadequate amounts, of vitamin A for normal growth.

Symptoms, Course, and Mortality.—The symptoms in poults receiving very minute quantities of, or no vitamin A, from the time of hatching were those of an acute-infectio-contagious disease except that fever was absent. In the initial experiment the symptoms were first noticed on the 25th day after hatching; in the second experiment, on the 26th day. The first death occurred on the 30th and 32nd days, respectively, and 100 per cent mortality by the 44th and 40th days, respectively. In contrast, the chicks used for comparison first showed symptoms on the 27th day, the first death occurred on the 34th day, and all were dead by the 56th day.

Daily notes from the first appearance of symptoms were taken on 50 poults. These birds displayed listlessness and an unsteady gait (always the first symptoms observed); they tended to sit with sagging wings,

·drooping heads, and closed eyes (fig. 1). Other early symptoms were increased lacrimation, suggested by "foaming" of the lacrimal secretions; swelling of the nictitating membranes; and a slight nasal discharge. In 4 of the 50 poults, symptoms were either not observed or were just appearing at the time of death.

Since the basis for daily individual examination was the discovery of definite abnormalities by flock inspection, such symptoms as swollen nictitating membranes may have been overlooked until the poult was picked up for examination. A milky exudate, though occasionally present early, was usually a later symptom. As the disease advanced, the nictitating membrane often concealed half the eyeball and appeared dry and rough, the surface sprinkled with a finely divided white powdery exudate.

Fig. 1. A five-weeks-old poult and a six-weeks-old chick. Both received the basal ration from time of hatching and were showing typical symptoms of A-avitaminosis for the respective species.

Early in the morning, many poults were found with their eyes "glued" shut. Eye changes were observed in 46 of the 50 poults during the course of the disease. In some, as the disease advanced, the secretion tended to increase rather than decrease and to appear milky. Little or no caseated whitish-yellow pus formed in the eyes or sinuses, probably because the disease was acute.

In only 1 poult were pustules in the mouth, common in more chronic cases, observed before death. Slight nasal discharges were noted in 22 cases, and marked nasal catarrh appeared in 6. In these, the poults tended to sneeze and cough, though such symptoms were not general. The sick poults continued to eat and drink until just before death. When symptoms first appeared, it was very difficult to keep water fountains clear of food deposited there because of the difficulty in swallowing. No similar trouble was experienced in the control pens or in pens that failed to show symptoms.

Rectal temperatures continued within the normal range, 105.0° to 107.0° F, until just before death, when they took a marked drop to as low as 101.4°.
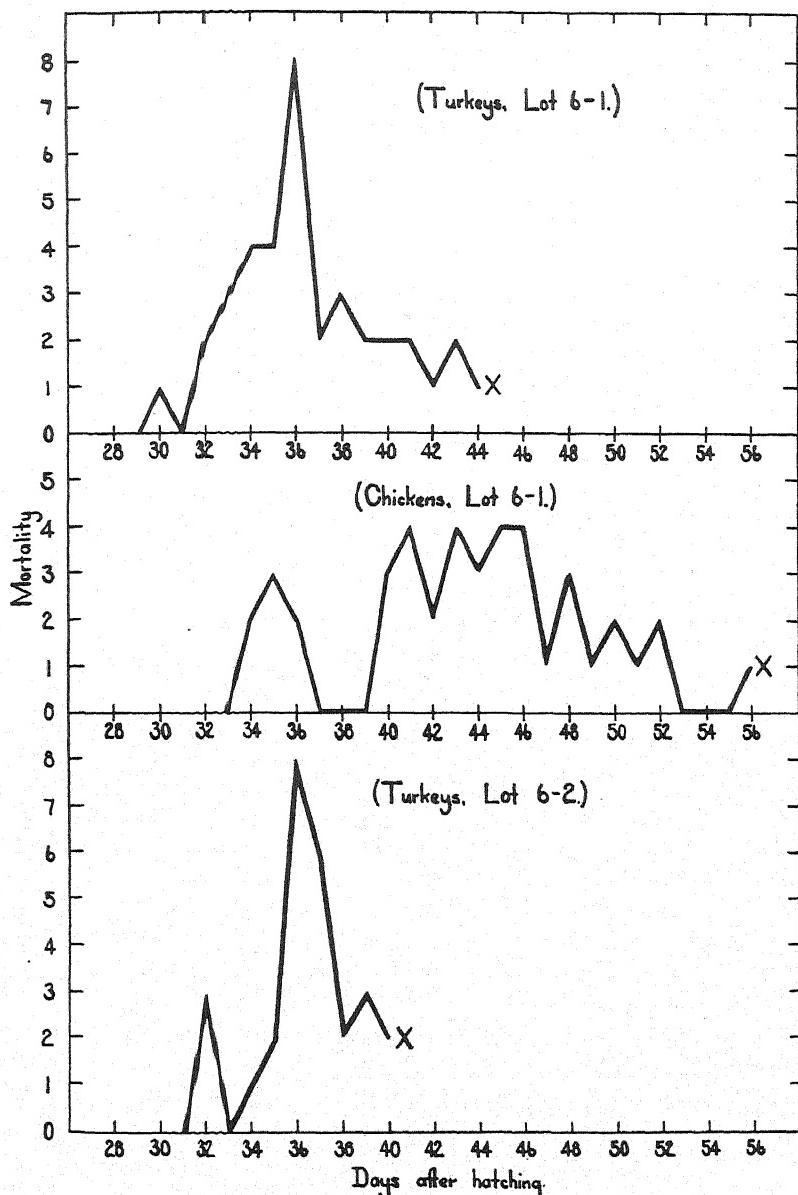
Fig. 2. Comparative mortality rates for the chicks and poults receiving the basal ration. No losses from A-avitaminosis occurred before the 30th day, and 100 per cent mortality was reached at the time indicated by *X*.

Figure 2 shows the mortality curves of the two pens of poults and the one pen of chicks from the 28th day until all the birds were dead. Among the chicks there was no mortality until losses from A-avitaminosis began. Five turkeys in one pen and 2 in the other died from undiagnosed causes before definite lesions of A-avitaminosis were observed. In each case the turkey mortality curve resembled that of an acute infection, with most deaths occurring on the 4th and 6th day after symptoms first appeared.

TABLE 1

DEATHS IN LOT 6 (BASAL RATION) DISTRIBUTED BY DAYS FROM FIRST APPEARANCE
OF SYMPTOMS IN THE FLOCK TO TIME OF DEATH

Number of days to death after first appearance of symptoms	Turkeys		Chickens	
	Number of deaths	Per cent of total deaths	Number of deaths	Per cent of total deaths
1	7	12.96	0	0.00
2	4	7.41	0	0.00
3	14	25.93	0	0.00
4	11	20.37	2	4.76
5	4	7.41	2	4.76
6	5	9.26	2	4.76
7	7	12.96	3	7.14
8	0	0.00	5	11.90
9	1	1.85	3	7.14
10	1	1.85	5	11.90
11	0	0.00	5	11.90
12	0	0.00	4	9.52
13	0	0.00	4	9.52
14	0	0.00	1	2.38
15	0	0.00	2	4.76
16	0	0.00	3	7.14
17	0	0.00	1	2.38

Of 5 poults observed while dying, 4 lay on their sides in a comatose condition and, except for gasping and slightly increased movement of the legs, did not struggle. The fifth struggled and flapped its wings until it finally gasped and died.

The symptoms, the course of disease, and the rate of mortality in the chicks receiving the basal ration were more chronic than in the turkeys on the same ration. The chicks, like the turkeys, became dull and listless. When disturbed, however, they were nervous and excited, a condition not observed in the turkeys.

Records on the course of the disease were kept on 54 poults and 42 chicks. Table 1 gives the distribution of deaths according to the time after appearance of first symptoms. As shown by this table and by figure 2, the course of the disease was much more prolonged in the chicks.

Gross Pathology.—Each bird that died was carefully examined for

gross pathological changes, fungi, coccidia, and worm eggs. Bacteriologic examinations were made on 20 poults and 18 chicks, with results that will be reported under another heading.

The types and principal location of the gross lesions are given in table 2. Most of the birds were in fair flesh at the time of autopsy, and a few that died early in the experiment were in good condition.

TABLE 2

DISTRIBUTION OF PRINCIPAL LESIONS IN TURKEYS AND CHICKENS IN LOT 6
(BASAL RATION)

Type and location of lesions	Turkeys*		Chickens†	
	Number	Per cent	Number	Per cent
Xerophthalmia	52	81.25	42	100.00
Sinusitis	29	45.31	13	30.95
Pustules in mouth	21	32.81	34	80.95
Pustules in upper esophagus	24	37.50	23	54.76
Pustules in crop	39	60.94	32	76.19
Pustules in lower esophagus	25	39.06	17	40.48
Enlarged proventriculus	2	3.13	11	26.19
Catarrhal to caseous exudate in bursa of Fabricus	44	68.75	37	88.10
Excessive urates in kidneys	6	9.38	25	59.52
Excessive urates in ureters	1	1.56	25	59.52
Urates in abdominal cavity	0	0.00	10	23.81
Urates in thoracic cavity	2	3.13	8	19.05

* Total turkeys observed, 64.
† Total chickens observed, 42.

As shown in table 2, the lesions were similarly located in the two species but with one exception (sinusitis) were somewhat more prevalent in the chick. This was especially true of urate deposits, which are considered of diagnostic value in A-avitaminosis of chickens. In only 6 of the 64 poults examined were abnormal urate deposits observed in the kidneys, and in only 1 were the ureters abnormally distended. In contrast, 25 of the 42 chicks had enlarged kidneys and ureters distended with urates.

The bursa of Fabricus is not mentioned, in the A-avitaminosis literature available, as an organ commonly affected in the disease of young birds. In these experiments, however, 44 of the 64 turkeys and 37 of the 42 chicks exhibited lesions in it. Most commonly, the bursa contained a deposit of a flaky white urate-like substance in varying quantities, which often distended it to two or three times its normal size (fig. 3). In other cases, the lesions varied from a thick mucoid exudate to a mixture of white flaky and mucoid deposits. In most instances the mucous membranes appeared dry but were not greatly involved.

To determine the incidence of such deposits in normal birds, between 200 and 300 young turkeys known to have been receiving adequate

amounts of vitamin A have been examined. In no instance has a similar deposit been observed in the bursa.

Pustules, containing a whitish-yellow caseous exudate, in the glands of the upper digestive tract, were much more pronounced in chicks than in poults. In the latter, the glandular portions of the crops showed lesions more consistently than the other divisions, but in most instances ex-
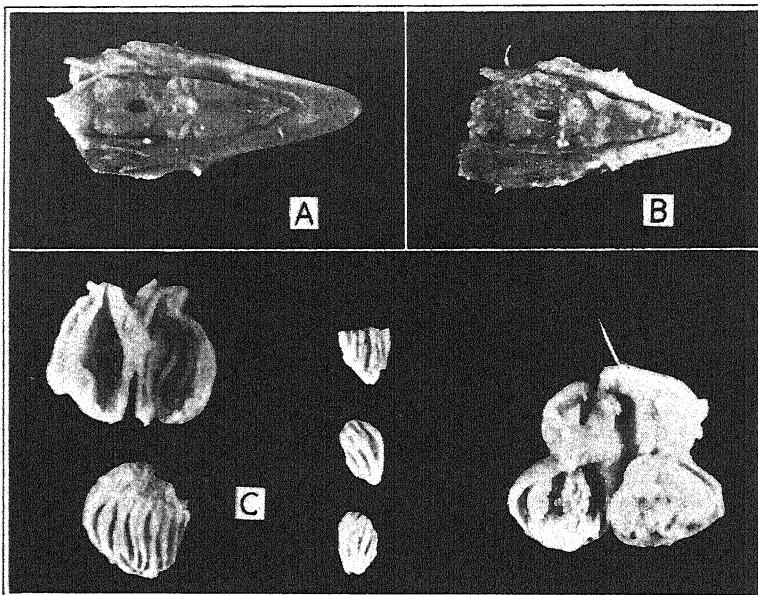
Fig. 3. *A,* Floor of mouth and pharyngeal region of a 40-day-old turkey that died from A-avitaminosis.

B, Same of a 45-day-old chick. Note the large numbers of pustules in *B* as compared with *A.* These specimens are typical for the two species.

C. Sagittal sections of bursas of Fabricus and caseous plugs characteristic of A-avitaminosis in young turkeys and chickens. The left bursa was from a poult; the right, from a chick. The middle specimens are typical caseous plugs from bursas taken from chicks.

hibited only swelling. Seldom were more than five or six pustules observed in the mouth or esophagus of the poults, whereas in the chicks the mucous membranes were frequently studded with lesions. Figure 3 shows the caseous deposits in the bursa of Fabricus and the swollen mouth glands of a chick and a poult.

The infraorbital and orbital sinuses were commonly involved in both species, but more severely in the poults. In the acute cases where few or no nasal discharges were noticed, the mucous membranes of the sinuses were inflamed and contained little or no exudate. In birds that lingered

longer before dying, a deposit of whitish-yellow flaky exudate was often found.

The proventriculus was enlarged in 11 chicks and 2 turkeys. In these cases, the glands were swollen and filled with a milky exudate, but no caseation appeared.

Intestinal changes were not consistent. Slight lack of tone and catarrhal inflammation of the duodenum were seen in a few of the turkeys and chickens. In some of the chicks the cloacae were filled with a granular-to-flaky urate deposit.

A few coccidia were demonstrated in cecal scrapings from 1 poult, and tapeworms in 1 chick. Otherwise, the birds were free of all parasites, and no fungi were found in any crop or esophagus.

Bacteriologic Findings.—Seedings on turkey meat infusion agar were made with the bone marrow and with either liver tissue or heart blood from each of 20 poults and 18 chicks. These cultures were incubated for at least 72 hours at 37° C.

Escherichia coli was isolated in pure culture from the bone marrow of 1 turkey and from the heart or liver of 7 turkeys and 1 chick. *Pseudomonas aeruginosa* was isolated from the bone marrow of 1 chick and from the heart or liver of 11 chicks and 1 poult.

EFFECT OF LOW VITAMIN-A LEVELS

The effect of low vitamin-A levels on the health of birds has probably more practical importance than has the effect of completely deficient vitamin-A rations. Certain field evidence indicates that hypovitaminosis (border-line cases) not showing typical recognized lesions may cause considerable losses. Lots 1 to 5 were included in order to ascertain the manifestations of hypovitaminosis in turkeys. Chickens were used in one trial, as mentioned previously, for the purpose of comparison. All pens were held intact for 28 to 30 weeks.

Since no clinically recognized cases of A-avitaminosis occurred in any chickens receiving more than 1 per cent of alfalfa meal, such groups can be eliminated. No mortality from A-avitaminosis occurred in the chickens receiving 1 per cent of alfalfa meal, although a few clinical cases developed towards the end of the experiment. Being fairly typical of cases of A-avitaminosis already described, these will not be included in this paper.

The results with turkeys will be discussed collectively for the two trials, from the standpoint of the relation to other diseases and of A-avitaminosis manifestations.

Influence on Other Disease Conditions.—Table 3 summarizes the probable causes of death for both trials. A-avitaminosis in each case was diagnosed by observing symptoms before death and by autopsy findings. With few exceptions, the birds were not killed, but allowed to die, before autopsy. Other diseases and pathological changes are included because in this phase of the experiment the complicating factors are probably as important as A-avitiminosis. All pens had the same oppor-

TABLE 3

DISTRIBUTION OF DEATHS IN TURKEYS ACCORDING TO CAUSE

Probable cause of death	Lot 1 (Basal ration plus green alfalfa)	Lot 2 (Basal ration plus 8 per cent dehydrated alfalfa-leaf meal)	Lot 3 (Basal ration plus 4 per cent dehydrated alfalfa-leaf meal)	Lot 4 (Basal ration plus 2 per cent dehydrated alfalfa-leaf meal)	Lot 5 (Basal ration plus 1 per cent dehydrated alfalfa-leaf meal)	Lot 6 (Basal ration only)
A-avitaminosis	0	0	11	17	11	64
A-avitaminosis and coccidiosis	0	0	6	15	29	1
A-avitaminosis, coccidiosis, and mycosis of crop	0	0	1	9	1	0
A-avitaminosis and mycosis of crop	0	0	3	6	0	0
Coccidiosis	3	4	1	2	0	0
Paralysis plus coccidiosis	4	4	0	0	0	0
Paralysis of very young poults without coccidiosis	0	3	4	1	0	0
Enteritis	23	2	4	3	0	0
Miscellaneous	6	5	3	4	1	6
Total mortality	36	18	33	57	42	71
*Per cent mortality**	*50.70*	*25.35*	*46.48*	*80.28*	*100*	*100*

* Based on 71 turkeys used in each lot except in lot 5, where 42 were used.

tunity for spread of infections; and, according to the evidence, in both coccidiosis and moniliasis, vitamin-A deficiency may be a factor in the mortality. Proof of this observation, however, lies in artificially producing the diseases in question so as to have equal distribution in all pens.

The excessive mortality caused by enteritis in the pens receiving green alfalfa (lot 1) occurred during the first 6 weeks and was greatest during the first 3 weeks. This loss has not been accounted for, although there is some field evidence that very young poults apparently do not tolerate freshly chopped young alfalfa leaves given earlier than the second week after hatching.

Part of the mortality not associated with gross lesions of A-avitaminosis is traceable to the fact that the experiments were conducted at the end of the regular breeding season; late-hatched poults in the hot interior valleys of California often suffer considerable infant mortality.

Coccidiosis did not appear in either experiment until the poults were about 8 weeks old.

Moniliasis of the crop was an important factor in lots 3 and 4 of the second experiment, in which no lot 5 was included. Other research work by one of the authors (Hinshaw[7]) has shown that moniliasis in turkeys has most economic importance in flocks suffering from other debilitating conditions.
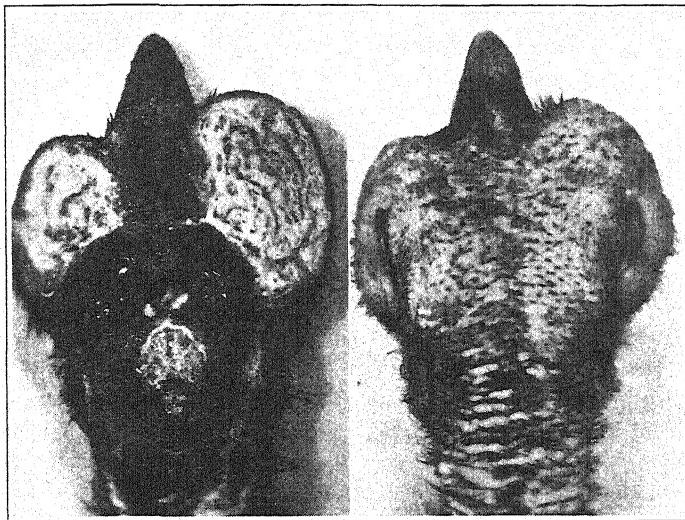
Fig. 4. An extreme case of sinusitis in a turkey hen suffering from A-avitaminosis after being fed for 8 months on a ration containing a low level of vitamin-A. The picture on the left is a sagittal section of the head shown on the right. Note the massive accumulation of the whitish-yellow caseous exudate typical of sinusitis as associated with A-avitaminosis in turkeys.

Tapeworms *(Choanotaenia infundibulum)* were somewhat more common in the chickens than in the turkeys killed for autopsy at the close of the experiment; and they were most prevalent in the chickens receiving a 1 per cent level of alfalfa meal.

Sinusitis (swellhead) of unknown etiology is prevalent in many turkey flocks. Field observations have sometimes suggested vitamin-A deficiency as a contributing factor; this possibility was further confirmed in a feeding trial conducted in 1931. Two lots of 38 Bronze turkeys each were started June 24, 1931, on the following "all-in-one" mash as a basal ration: 25 pounds yellow corn, 25 pounds wheat, 25 pounds barley, 15 pounds fish scraps, 10 pounds dried milk, 5 pounds bone meal, 2 pounds limestone, 0.5 pound sodium chloride.

Both lots were cared for identically except that one had daily access to

fresh greens (lawn clippings or freshly chopped alfalfa) whereas the other had 5 pounds of sun-cured alfalfa meal (in this instance, leaf and stem meal) added to the basal ration and received no fresh greens. Both lots appeared normal for about 4 months; then the one receiving the alfalfa meal became unthrifty. The green-feed lot made normal gains until the termination of the experiment on March 9, 1932. The total mortality in the two groups was 78.94 and 20.51 per cent, respectively.
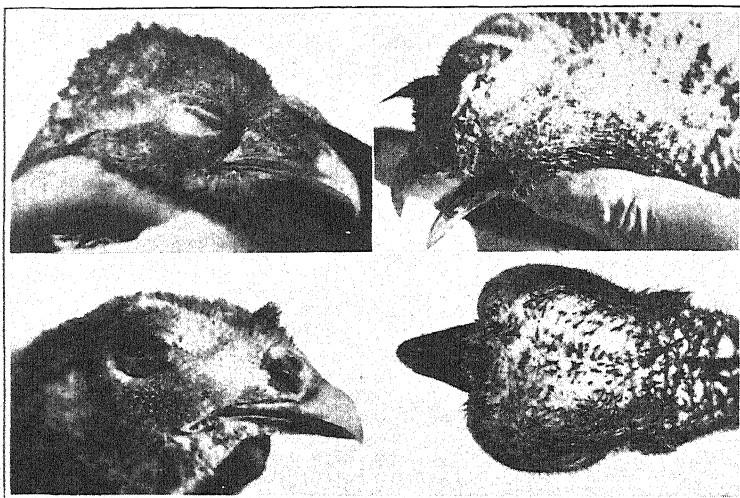
Fig. 5.—Four turkey heads photographed a few days before the birds died from A-avitaminosis. These illustrate the various degrees of ophthalmia and sinusitis seen in the advanced stages of the more chronic form of A-avitaminosis, caused by a continuously low vitamin-A diet.

Apparently the sun-cured alfalfa meal used was of low vitamin content, for by December 18 a few typical cases of xerophthalmia had appeared among the turkeys receiving this supplement. Before the close of the experiment more than one-half of the turkeys in this lot developed sinusitis, in its early stages indistinguishable from swellhead (fig. 4). Typical lesions of A-avitaminosis were found in the birds that died. In contrast, the other group remained normal and showed no indication of A-avitaminosis on postmortem examination of representative birds.

In the 1932–33 experiments, individual cases of sinusitis indistinguishable in the early stages from swellhead were seen, but they were less severe than in 1931–32 (fig. 5).

Comparison of A-Avitaminosis Manifestations in the Different Lots.— The disease in the turkeys of lot 6, which received no vitamin A, was so acute that in many instances manifestations of A-avitaminosis did not

TABLE 4

SUMMARY OF MORBIDITY AND MORTALITY IN THE TURKEYS ASSOCIATED
WITH A-AVITAMINOSIS

Lot and ration		Cases of A-avitaminosis		Deaths associated with A-avitaminosis		Days to death from first symptoms	
		Total number	Per cent of total birds*	Total number	Per cent of total birds*	First death	Last death
Lot 3 (Basal ration plus 4 per cent of dehydrated alfalfa-leaf meal)	First trial	7	16.67	7	16.67	63	32 alive at termination
	Second trial	16	55.17	14	48.28	42	6 alive at termination
Lot 4 (Basal ration plus 2 per cent of dehydrated alfalfa-leaf meal)	First trial	28	66.67	25	59.52	43	11 alive at termination
	Second trial	23	79.31	22	75.86	34	5 alive at termination
Lot 5 (Basal ration plus 1 per cent of dehydrated alfalfa-leaf meal)†		41	97.62	41	97.62	47	147‡
Lot 6 (Basal ration)	First trial	38	90.48	38	90.48	30	44‡
	Second trial	27	93.10	27	93.10	32	40‡

* Based on 42 birds in first trial and 29 birds in second trial.
† Only one trial with lot 5.
‡ All died.

TABLE 5

SUMMARY OF DISTRIBUTION OF GROSS LESIONS IN THE TURKEYS

Location of lesions	Lot 3 (Basal ration plus 4 per cent of dehydrated alfalfa-leaf meal)		Lot 4 (Basal ration plus 2 per cent of dehydrated alfalfa-leaf meal)		Lot 5 (Basal ration plus 1 per cent of dehydrated alfalfa-leaf meal)		Lot 6 (Basal ration only)	
	Birds affected	Per cent of total	Birds affected	Per cent of total	Birds affected	Per cent of total	Birds affected	Per cent of total
Eyes	15	71.43	31	68.89	37	90.24	52	80.00
Sinuses of head	3	14.29	8	17.78	5	12.20	29	44.62
Mouth	4	19.05	13	28.89	31	75.61	21	32.31
Upper esophagus	5	23.81	16	35.56	31	75.61	24	36.92
Crop	2	9.52	9	20.00	26	63.41	39	60.00
Lower esophagus	6	28.57	15	33.33	29	70.73	25	38.46
Kidneys	4*	19.05	0	0.00	0	0.00	6	9.23
Bursa of Fabricus	8	38.10	16	35.56	37	90.24	44	67.69
Trachea	2	9.52	5	11.11	25	60.98	0	0.00

* Very slight indication of excessive urates.

appear. The longer the birds suffering from inadequate supplies of vitamin A lived, the more typical the disease was of that seen in chickens. This was especially true regarding the external symptoms and lesions.

Table 4 summarizes the data collected when the first recognizable symptoms appeared, and the average time of death after appearance of first symptoms. Lot 6 is included for comparative purposes.

The total period for each trial was between 210 and 220 days. Apparently the amount of vitamin A in the ration and the time of the first
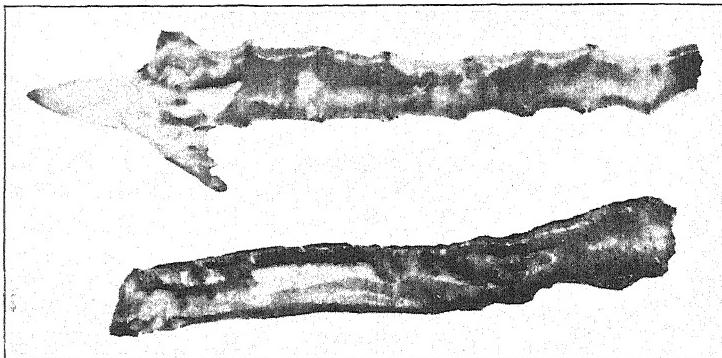
Fig. 6. Trachea of turkey No. 1094, lot 5 (basal ration plus 1 per cent of dehydrated alfalfa-leaf meal). Died from A-avitaminosis at the age of 71 days. Note the caseous plugs at the superior laryngeal opening and the tube-like casts, posterior. Both were common types of lesions found in the more chronic cases in the low-level vitamin-A groups.

death associated with A-avitaminosis were not correlated; but frequently the lack of vitamin A was not the primary cause of death, although lesions were found on autopsy. On the other hand, the course of the disease, the total number of cases of A-avitaminosis, and the percentage mortality were directly related to the vitamin-A level in the rations.

A marked individual variation in susceptibility of the turkeys to A-avitaminosis was noted in lots 3, 4, and 5. This may be attributed to the selective ability of certain birds to obtain more vitamin A than others, or to natural resistance. The most notable example of this individual variation occurred in lot 5: a male, D 1037, lived 147 days (46 days longer than its penmates) and held its own weight for over a month after the others were dead.

Distribution of Lesions.—Table 5 summarizes the distribution of gross lesions in the turkeys suffering from A-avitaminosis. These data were secured only from cases that died or were killed in coma.

The distribution of lesions was not markedly different in birds receiving various levels of vitamin A. Attention is again called to the infrequent occurrence of kidney lesions. A smaller percentage of bursa-of-

Fabricus lesions might be expected in lots 3 and 4, for these birds were older when the losses started, the bursal opening had often closed, and the bursa itself was more or less atrophied. Tracheitis, ranging from a
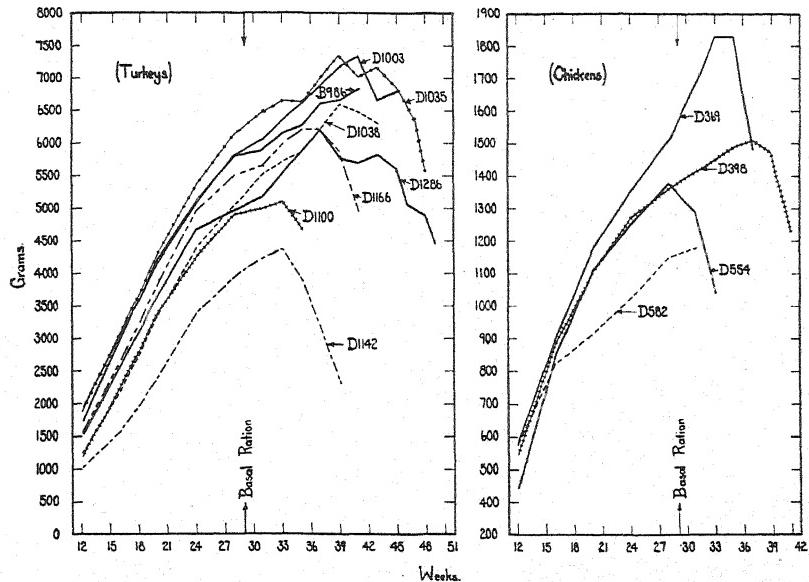
Fig. 7. Growth rates of representative turkeys and chickens fed on a vitamin-A-free diet after first receiving various levels of vitamin A for 29 weeks. The original rations for the different birds were as follows: Turkeys D 1100 and D 1142, basal plus 2 per cent of dehydrated alfalfa-leaf meal; B 986 and D 1166, basal plus 4 per cent of dehydrated alfalfa-leaf meal; D 1003 and D 1035, basal plus 8 per cent of dehydrated alfalfa-leaf meal; D 1038 and D 1286, basal plus fresh greens; chickens D 554 and D 582, basal plus 1 per cent of dehydrated alfalfa-leaf meal; and D 369 and D 398, basal plus 2 per cent of dehydrated alfalfa-leaf meal.

catarrhal to a croupous type, was prevalent in many cases, especially in lot 5. Figure 6 shows a typical case of the latter type.

Urate deposits on the heart or viscera were so uncommon that they were not included in the table.

EFFECT OF REMOVAL OF VITAMIN A FROM THE RATIONS

The phase of the experiments discussed in the following section was conducted to ascertain the length of time required to deplete the body storage of vitamin A, and the disease manifestations under such conditions.

Three representative turkeys from each of the pens of lots 1, 2, 3, and 4, and three representative chickens from each of lots 3 and 4 were placed in the same pen on January 11, 1933, with the basal ration as their sole diet. Daily observations were made for symptoms of A-avitaminosis, and weights were taken of each individual, first at two weeks'

intervals, later at weekly intervals, until death. The growth data for these birds are given in figure 7.

The time of first symptoms and the survival time were directly correlated with the vitamin-A storage, as indicated both by the amount of alfalfa meal in the original rations and by the number of antimony tri-

TABLE 6

RELATION BETWEEN VITAMIN-A STORAGE IN THE LIVERS OF REPRESENTATIVE TURKEYS AND CHICKENS KILLED AT 30 WEEKS OF AGE AND THE SURVIVAL TIME OF PENMATES PLACED ON THE BASAL RATION*

Lot numbers and original ration	Vitamin-A units per gram of liver at 30 weeks	Days after placing on basal ration to		
		First clinical symptoms	First death	Last death
Lot 1, turkeys (basal ration plus green alfalfa)	100	96	†	140
Lot 2, turkeys (basal ration plus 8 per cent of dehydrated alfalfa-leaf meal)	65	87	103‡	135
Lot 3, turkeys (basal ration plus 4 per cent of dehydrated alfalfa-leaf meal)	30	56	90	¶
Lot 4, turkeys (basal ration plus 2 per cent of dehydrated alfalfa-leaf meal)	1	33	53	86
Lot 4, chickens (basal ration plus 2 per cent of dehydrated alfalfa-leaf meal)	36	56	60	85
Lot 5, chickens (basal ration plus 1 per cent of dehydrated alfalfa-leaf meal)	4	14	24	37

* Table data from work published elsewhere. (5)

† Two birds killed to determine the antimony trichloride blue value at the time of first clinical symptoms.

‡ One bird killed on the 96th day which probably would not have lived to the 107th day.

¶ Remaining birds at 90 days were in advanced stages of deficiency and were autopsied.

chloride blue units in liver extracts in a representative number of penmates killed before this group was placed on the basal ration. Table 6 summarizes these data.

Considerable individual variation was noted, being especially marked in the lot 4 group of turkeys. There was a difference of 33 days in the time of the first and the last death in this lot. The last survivor lived as long as did one of the chickens receiving an equal supply of alfalfa in the original ration, but the chickens on an original 2 per cent level were more comparable to the turkeys on the 4 per cent level.

Figure 8 shows the typical attitude of a turkey that developed A-avitaminosis after being placed on a vitamin-A-free diet. Figure 9 shows the type of lesions seen in the upper esophagus in such cases.
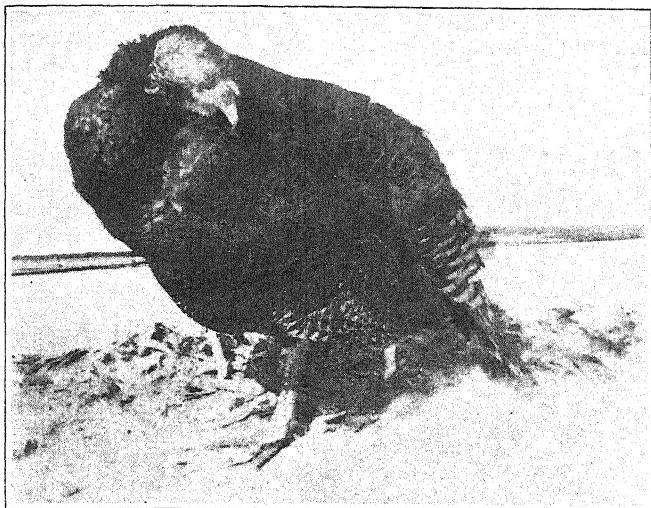
Fig. 8. Turkey hen No. 1166, taken 87 days after transfer from a ration containing a 4 per cent level of dehydrated alfalfa-leaf meal to the basal ration. The bird died 3 days later.
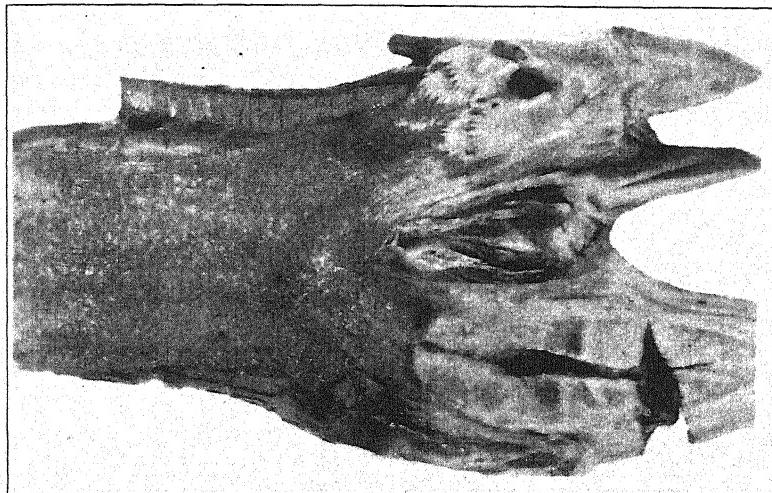
Fig. 9. Portion of head and esophagus of turkey hen No. 1166 (fig. 8) laid open to show the pustular lesions in this part.

MINIMUM VITAMIN-A REQUIREMENTS

Figure 10 presents the growth data for the 1932–33 experiments. According to these data and table 3, the turkeys required at least 8 per cent of alfalfa meal to prevent A-avitaminosis. Furthermore, according to the data on chickens, turkeys require considerably more vitamin A for
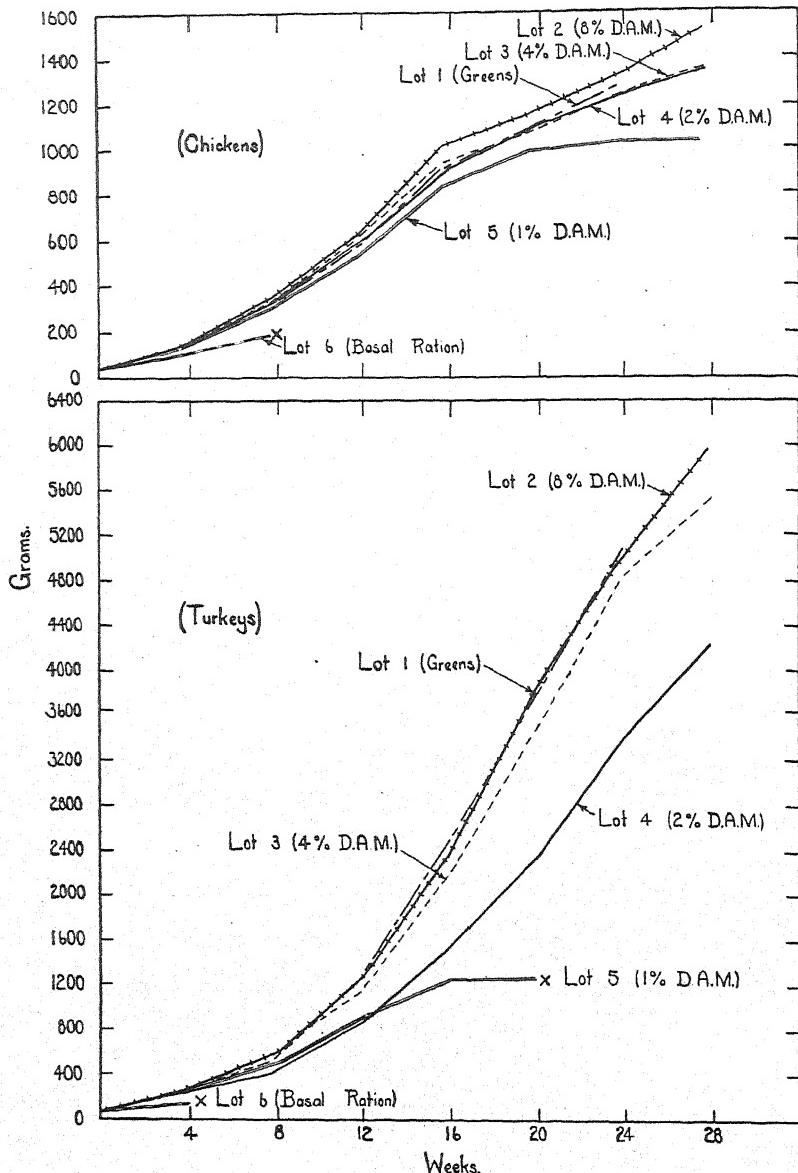
Fig. 10. Growth rates of chickens and turkeys, lots 1–6, inclusive. (The abbreviation "D.A.M." refers to dehydrated alfalfa-leaf meal.)

normal growth. These conclusions are further substantiated by liver analysis (table 6).

The amounts of freshly cut alfalfa consumed by the pens receiving their vitamin A from this source were recorded. In the first trial, includ-

ing both turkeys and chickens, the average consumption in terms of dried alfalfa meal[6] was 11.13 per cent of the total feed consumed. In the second trial, including only turkeys, the average consumption was 9.51 per cent of the total ration.

Table 7 summarizes the estimated consumption by 4-week growing periods. Differences in the two trials probably arise from varying consumption in the individual pens rather than from the influence of the chickens in the first trial.

TABLE 7

PERCENTAGE OF FRESH ALFALFA FED LOT 1 CONVERTED TO PERCENTAGE OF DRIED ALFALFA, IN WHOLE RATION*

	Growth periods						Average for total period
	0–4 weeks	5–8 weeks	9–12 weeks	13–16 weeks	17–20 weeks	21–24 weeks	
Trial 1, chickens and turkeys	2.61	9.83	12.38	10.84	11.20	12.27	11.13
Trial 2, turkeys only..............	3.01	8.00	8.38	10.04	10.17	10.36	9.51

* Calculated on the basis of fresh alfalfa of the age used, containing 75 per cent moisture.

In both trials, the percentages of dried alfalfa in terms of the total feed consumption with one exception increased with the age of the birds. The amounts consumed further indicated that a ration containing 8 per cent of a good grade of alfalfa meal, like that used in lot 2, approaches the optimum amount for normal growth of turkeys when further vitamin A is not supplied. The data obtained on vitamin-A storage in the livers of these birds (table 6) indicated that they had stored slightly more vitamin A than those receiving 8 per cent of alfalfa meal. The growth rates of the two groups were, however, almost identical (fig. 10).

DISCUSSION

The purpose in outlining these studies was to determine the possible relation of A-avitaminosis to obscure losses in range-reared turkey flocks. These studies showed that certain disease manifestations of A-avitaminosis in turkeys were more obscure than those in chickens under the same conditions. Since a careful search for A-avitaminosis lesions was often required when specimens were brought to the laboratory after death, routine autopsy might have failed to detect the lesions in many instances. For this reason, a diagnostician should inquire carefully into the feeding history and should examine the sinuses, the eyes, and the

6 Based on unpublished data by H. R. Guilbert, who found that freshly cut alfalfa, of the approximate maturity of that used, contains about 75 per cent moisture.

upper digestive tract for evidences of the disease. Uremia, as indicated by urate deposits on the viscera or by enlarged kidneys and ureters with excessive urate deposits, was seldom seen, even in the more chronic cases, and cannot be considered diagnostic. The bursa of Fabricus, on the other hand, if found thickened and containing a white flaky deposit between the folds, suggests A-avitaminosis.

Scott[7] called attention to hemorrhagic enteritis as a common lesion in the poults of his low-vitamin-diet groups that died from A-avitaminosis. A recheck of our autopsy reports shows that only three specimens of 70 examined (all of which were uncomplicated with coccidiosis or other parasites) were suffering from mild hemorrhagic enteritis. About half of the poults had irregular areas of congestion in the upper third of the intestine, but catarrhal enteritis was a common manifestation.

The results obtained with the Carr-Price[2] antimony trichloride color reaction, utilized in a part of these studies, suggested that it might be an aid in diagnosing borderline cases of A-avitaminosis. The results reported by Guilbert and Hinshaw[6] indicate a direct relation of vitamin-A storage in turkey livers to the age of the bird. Experiments now under way may yield further data on vitamin-A storage at different ages, which will serve as criteria for diagnosing borderline cases of vitamin-A deficiency.

Vitamin A is generally recognized as important in disease prevention, especially in diseases of the head and respiratory organs (Green[5], Turner and Loew[11]). As already noted, at least two important infectious diseases appeared in the 1932–33 trials—coccidiosis and moniliasis. Neither disease occurred among the chickens in the same pens, nor in the turkeys until after the poults and chicks fed on the basal ration had all died. In lots 3, 4, and 5, according to the evidence obtained, inadequate levels of vitamin A may have influenced the severity of the two diseases, since the turkeys in lots 1 and 2 had equal opportunity to contract the same infections.

The reason for the chickens' not developing coccidiosis was that chicken types of coccidia were not present (as determined by frequent fecal examinations) until a few weeks before the close of the experiment. Although chickens are susceptible to moniliasis (Jungherr[8], Hinshaw[7]), they did not contract the disease, presumably because they received a more adequate diet.

Several outbreaks of sinusitis indistinguishable from the disease commonly called swellhead have been observed in connection with vitamin-A deficiency and have responded to cod-liver oil treatment. On the other

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[7] Scott, H. M., in personal interview, August, 1934.

hand, numerous outbreaks of equal severity have occurred on ranches where the turkeys have had ample vitamin A in the form of alfalfa meal and fresh greens.

The experimental and field evidence that in certain outbreaks swellhead is definitely related to vitamin-A deficiency, however, suggests the need for more research on this subject. The available knowledge of this relation emphasizes, furthermore, the need of eliminating the possibility of vitamin-A deficiency in all outbreaks of swellhead.

In the 1932–33 experiments, generalized bacterial infections were uncommon. *Escherichia coli* predominated in the turkeys that yielded organisms from the livers, heart bloods, and bone marrow, while *Pseudomonas aeroginosa* was most often isolated from the chickens. This latter observation is especially interesting because Green[5] reported spontaneous *Ps. aeroginosa* infection of rabbits suffering from experimental A-avitaminosis, but not in the controls.

Elvehjem and Neu[4] produced symptoms in White Leghorn chicks with their basal ration somewhat sooner than was possible with the basal ration used in these experiments. This difference may have resulted partly from a slight amount of vitamin A in the latter basal ration, from the small amount of water-grass seed in the rice hulls used as litter, or from a greater reserve storage in the chicks at the time of hatching. Since the poults were kept in the same pens with the chicks, the differing results obtained with the two species are considered comparable.

The results reported indicate that the severity of coccidiosis and moniliasis was influenced by the amounts of vitamin A received by the various groups. Thus they emphasize the importance of making complete autopsy examinations in such experiments, because the practical results of A-avitaminosis are produced not only by the disease itself, but by its effect on existing parasites, infective organisms, and other debilitating conditions.

SUMMARY AND CONCLUSIONS

A comparative study of A-avitaminosis in Bronze turkeys and White Leghorn chickens is reported.

Poults fed a vitamin-A-free ration from the time of hatching, developed symptoms of A-avitaminosis in 25 days in one trial, and in 26 in a second trial. Chicks kept as penmates to the poults in one of these trials began to show symptoms on the 27th day. The disease was much more acute among the poults than among the chicks, the first death among the former occurring on the 30th and 32nd days, respectively, with 100 per cent mortality by the 44th and 40th days, respectively. The first death among the chicks occurred on the 34th day; the last, on the 56th.

In the poults, symptoms resembled those of an infectious disease. Xerophthalmia was the principal differential symptom noted in them as well as in the chicks; but caseated pus seldom collected in the eyes, as in older fowls. Marked nervousness was noted in the chicks but not in the poults.

Lesions in the poults were confined to mucous membranes of the head, the upper digestive tract, the respiratory tract, and the bursa of Fabricus. The lesions, though typical of those described in chickens, were never equally numerous and were confined principally to the crop and lower esophagus. Tracheitis was an occasional manifestation.

The bursa of Fabricus, not previously reported as a seat of lesions, was affected in 44 of 64 poults and in 37 of 42 chicks examined. A white, flaky, urate-like deposit between the thickened bursal folds was the most common manifestation.

Deposits of urates in kidneys and ureters, considered pathognomonic in diagnosis of A-avitaminosis in chicks, seldom occurred in the poults.

In the turkeys receiving various levels of dehydrated alfalfa-leaf meal as the source of vitamin A, the percentage mortality associated with A-avitaminosis varied inversely as the amount of alfalfa in the ration. It was 97.62 per cent, 66.19 per cent, and 54.93 per cent, respectively, for the lots receiving 1 per cent, 2 per cent, and 4 per cent levels of dehydrated alfalfa-leaf meal. No mortality associated with or caused by A-avitaminosis occurred in turkeys receiving either 8 per cent of dehydrated alfalfa-leaf meal or freshly cut alfalfa as the sole source of vitamin A.

Symptoms and autopsy findings in the turkeys dying in the low-level vitamin-A groups, though complicated with coccidiosis and moniliasis, did not differ essentially from those in the pens receiving no vitamin A. As in the vitamin-A-free groups a deposit of urates in the kidneys and ureters was not a common manifestation. The mortality associated with moniliasis and coccidiosis in the different lots indicated a possible relation between the severity of the diseases and the vitamin-A level of the rations.

Sinusitis, in some respects resembling a disease commonly called swellhead, was prevalent in the 1931–32 experiments and occasionally observed in 1932–33. This fact suggests the need of eliminating A-avitaminosis as a possible factor in all field outbreaks of this disease.

The survival time of representative turkeys and chickens, placed in a pen at the end of 30 weeks and given the basal ration, varied directly as the amount of vitamin A received before the transfer—from 24 days in chickens getting a 1 per cent level of dehydrated alfalfa-leaf meal in the

original ration, to 140 days for a turkey fed freshly cut alfalfa ad lib in the original ration.

Marked individual resistance was noted throughout the experiment. One poult receiving 1 per cent of dehydrated alfalfa-leaf meal lived a total of 147 days, or 46 days after its last penmate had died.

Bronze turkeys were found to require a ration including 8 per cent of dehydrated alfalfa-leaf meal (containing approximately 130 gamma of carotene per gram) for normal growth to 30 weeks of age. White Leghorn chickens kept as penmates to the turkeys made normal gains and showed no evidence of A-avitaminosis on as low as a 4 per cent level of the alfalfa meal.

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EXPERIMENTS WITH THE ASTER-YELLOWS
VIRUS FROM SEVERAL STATES[1]

HENRY H. P. SEVERIN[2]

INTRODUCTION

IN 1929 THE AUTHOR[10] reported that *Cicadula divisa* Uhl. [*C. sexnotata* (Fall.)] transmitted yellows from naturally and experimentally infected varieties of celery to asters and from asters to celery in California. Kunkel[5] failed to infect 9 varieties of celery with the aster-yellows virus from New York by means of *Cicadula divisa*. In later papers the author[11, 13] reported the transmission of yellows from naturally and experimentally infected varieties of carrot, parsley, and parsnip in California, but Kunkel[5] questions whether this disease is identical with aster yellows in New York, since the California aster-yellows virus is readily transmitted to celery and to *Zinnia elegans*, plants that are highly resistant if not immune to New York aster yellows.

Dorst,[1] who has made a study of the genus *Cicadula*, found that *Cicadula sexnotata* (Fall.) is a European species and that the American species is *Cicadula divisa* Uhl. Specimens of *Cicadula* were sent to Dorst by Kunkel from New York and by the writer from California, and all were determined as *Cicadula divisa*.

A review of the literature indicates that the celery yellows found in California probably occurs in other states. According to Linford,[7] aster and celery yellows first made its appearance in Utah during 1927.

Folsom[2] states that apparently the same disease as described by the author[10] was seen in southwestern Maine on an experimental farm,

where by systematic sweepings, the vector *Cicadula divisa* was caught for the first time in three years in which the work was carried on.

According to Vaughan and Foster,[19] aster yellows was found on celery in Wisconsin, but there was more infection in carrots than in celery or lettuce, when all three were growing adjacent to an experimental aster-yellows plot. Foster[3] planted about an acre of celery with asters between the rows at Madison, Wisconsin. He reports, "There were 3 celery plants in the entire field that developed symptoms that could be called typical aster yellows, without the twisting of the petioles. . . . In the same field I had a dozen large cages over celery plants in which colonies of *Cicadula divisa* were transferred early. This colony was collected from cages containing yellowed aster plants. Aster plants with yellows were also transplanted to the cages containing celery. These plants remained under the cages until late fall, and the hopper was present in large numbers at all times. None of the celery plants showed any of the yellows symptoms at any time."

Kunkel[6] experienced no difficulty in transmitting yellows to healthy aster and celery by means of *Cicadula divisa* from asters, celery, and carrots infected with California aster yellows, but failed to transmit the disease to zinnia. Yellows was also transferred from celery experimentally infected with California aster yellows, to healthy asters. California yellows on asters could not be distinguished by symptoms from the yellows disease prevalent on aster in New York. Kunkel came to the conclusion that celery yellows of California is not identical with aster yellows of New York.

Transmission experiments were performed, using *Cicadula divisa* occurring in California, with the aster-yellows virus obtained from New York, Indiana, and Wisconsin, carrot-yellows virus from Maine and Idaho, and celery-yellows virus from Utah, to determine whether healthy asters and celery could be experimentally infected with the disease. Tests were made to determine whether there are host-range differences between yellows viruses obtained from various states. Attempts were made to recover the virus from the experimentally infected plants. Experiments were also conducted to determine whether *Thamnotettix montanus* Van D., a newly discovered vector of California yellows, could transmit yellows from asters infected with the disease in New York and Wisconsin to healthy asters and celery. Attempts were also made to transmit yellows by feeding previously noninfective *Cicadula* on feeding solutions containing crushed infective leafhoppers which had fed on yellows-infected plants from New York and Wisconsin.

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[3] Foster, A. C., letter to author dated February 18, 1931.

ASTER-YELLOWS VIRUS FROM NEW YORK

Through the courtesy of L. O. Kunkel, Rockefeller Institute for Medical Research, Princeton, New Jersey, three shipments of asters and salsify infected with yellows were received in good condition from New York.

TABLE 1

TRANSMISSION OF NEW YORK ASTER YELLOWS TO HEALTHY ASTERS AND CELERY BY *Cicadula divisa**

Experiment 1			Experiment 2		Experiment 3	
Insects transferred from New York aster yellows to healthy celery	Same insects transferred from celery to healthy asters	Same insects transferred from infected asters to healthy celery	Insects transferred from experimentally infected asters to healthy asters	Insects transferred from experimentally infected asters to healthy celery	Insects transferred from experimentally infected asters to healthy asters	Insects transferred from experimentally infected asters to healthy celery
Infections resulting						
−	+	−	−	3−	−	5−
−	+	−	+	1+ 4−	−	5−
−	+	−	−	5−	−	5−
−	+	−	+	1+ 4−	−	5−
−	−	....	....		....	....
−	+	−	+	1+ 4−	−	5−
−	+	−	+	5−	−	5−
−	+	−	+	1−	+	1+ 4−
−	+	−	+	1−	−	5−
−	+	+	−	1−	+	5−
−	−	....	....		....	....
−	+	....	....		−	5−
−	+	....	....		+	5−
−	−	....	....			....
−	+	....	....		+	1+ 4−
−	−	....	....			....
−	+	....	....		−	5−
	13+	1+	6+	3+	4+	2+
17−	4−	8−	3−	28−	9−	63−

* The plus sign (+) indicates the production of the disease, and the minus sign (−) shows that no disease resulted.

Several experiments were conducted to determine whether asters, celery, carrots, and parsley could be experimentally infected with the aster-yellows virus from New York. In the first experiment previously non-infective male *Cicadula divisa* were fed on yellows-infected asters from New York for a period of 2 or 3 days and then 20 insects were transferred to each of 17 healthy celery plants. After feeding on the celery plants for a period varying from 19 to 27 days, each lot of leafhoppers was

transferred to a healthy aster plant. Ten of the 17 lots of leafhoppers, some of which had died, were again transferred from the inoculated asters to a second set of healthy celery plants for a period of 25 days. Table 1, experiment 1, shows that the first lot of celery plants remained healthy, 13 of the 17 asters inoculated became diseased, and 1 of the 9 celery plants in the second lot developed typical symptoms of yellows as described in a previous paper.[10] Celery plants used as a check or control remained healthy.

In the second experiment noninfective nymphs were exposed to 9 asters which had been experimentally infected with yellows in experiment 1. After the nymphs acquired the winged stage, lots of 5 to 35 males were transferred from each diseased aster to healthy asters and celery plants. Table 1 shows that in experiment 2, 6 of the 9 asters, and 3 of the 31 celery plants inoculated, showed typical symptoms of celery yellows.

In the third experiment 13 lots of leafhoppers after being exposed for a period of 8–28 days to 13 diseased asters from experiment 1, were transferred in groups of 5, to 13 healthy asters and 65 celery plants. Table 1, experiment 3, shows that 4 of the 13 asters and 2 of the 65 celery plants inoculated developed symptoms of yellows.

In the three experiments a total of 122 celery plants were inoculated with the New York aster-yellows virus, and 6 showed symptoms of celery yellows. The central leaves were chlorotic with a slight twisting of the petioles. Previously noninfective leafhoppers were exposed to the 6 celery-yellows plants and were then transferred to healthy asters and celery. The virus was repeatedly recovered by 3 lots of previously noninfective leafhoppers from only one celery-yellows plant, and typical symptoms of yellows developed with 3 asters. Previously noninfective leafhoppers, after feeding on the 3 diseased asters, failed to transmit yellows to 12 healthy celery plants.

It was decided to use larger numbers of leafhoppers with the salisfy infected with the aster-yellows virus from New York. Lots of 50 to 100 previously noninfective leafhoppers, after feeding on salsify infected with yellows for a period of 4 to 6 days, were transferred to successive healthy celery plants for periods of 2 weeks until all of the insects were dead. Eighty-five healthy celery plants were inoculated and 2 plants developed typical symptoms of yellows in the cages. Previously noninfective leafhoppers after feeding on the infected celery plants failed to transmit yellows to healthy asters and celery.

Carrots (*Daucus carota sativa*) showing symptoms of yellows have been reported by Whetzel,[20] Newhall,[8] and Kunkel[5] in New York, by Folsom[2] in Maine, by Zundel[21] in Pennsylvania, by Vaughan and Foster[19] in Wisconsin, and by the author[11, 13] in California.

An experiment was conducted to determine whether varieties of carrots could be experimentally infected with the yellows virus from asters infected in New York by means of *Cicadula divisa*. Previously noninfec-

TABLE 2

INCUBATION PERIOD OF NEW YORK ASTER YELLOWS IN EXPERIMENTALLY INFECTED
CARROTS AND RECOVERY OF VIRUS BY *Cicadula divisa*

Variety of carrot	Number of plants inoculated	Number of leaf-hoppers on each plant	Number of plants infected	Number of plants healthy	Incubation period in plant, days	Yellows transferred	
						From carrot to aster	From carrot to celery
Short White	1	20♂	1	0	15	—	—
	1	20♀	1	0	40	+	—
White Mastodon	1	20♂	1	0	40	—	—
	1	20♀	1	0	37	+	—
White Belgian	1	20♂	1	0	28	+	—
	1	30♀	1	0	18	+	—
Yellow Belgian	1	20♂	1	0	39	—	—
	1	30♀	1	0	53	—	—
Chantenay	1	20♂	1	0	24	—	—
	1	30♀	0	1	....	—	—
Danvers Half Long	1	20♂	1	0	39	+	—
	1	20♀	1	0	22	—	—
Early Scarlet Horn	1	20♂	1	0	21	—	—
	1	20♀	1	0	21	—	—
French Forcing	1	20♂	1	0	24	—	—
	1	30♀	1	0	42	—	—
Long Orange	1	10♀	1	0	16	—	—
	1	10♀	0	1	....	—	—
Nantes	1	20♂	1	0	39	+	—
	1	30♀	1	0	37	—	—
Oxheart or Guerande	1	20♂	1	0	28	—	—
	1	20♀	1	0	35	—	—
Total	22	....	20	2	....	6	0
Average	....	....	....	....	30.9	....	....

tive leafhoppers, varying in number from 10 to 30 males or females, were exposed to aster-yellowed plants and then transferred to 3 white, 1 yellow, and 7 orange varieties of carrots, as indicated in table 2.

It is evident from table 2 that 20 of the 22 carrots inoculated were experimentally infected with the New York aster-yellows virus. The virus was recovered by means of previously noninfective leafhoppers

from 6 experimentally infected carrots and transferred to 6 healthy asters. The virus was not transferred by previously noninfective leafhoppers from infected carrots to any of the 22 healthy celery plants inoculated. The incubation period of the disease in the plant varied from 15 to 53 days, with an average of 30.9 days.

Three Single or Plain parsley plants (*Petroselinum hortense*) were experimentally infected with the yellows virus from asters infected in New York. The infected parsley plants showed typical symptoms of yellows as described in a previous paper,[13] but the virus was not transferred by leafhoppers from the infected parsley plants to 8 healthy asters and 6 celery plants. The incubation periods of the disease in the plants were 30, 45, and 48 days, respectively, averaging 41 days.

Four Hamburg or Turnip-rooted parsley plants (*Petroselinum hortense radicosum*) were inoculated with the aster yellows virus from New York by means of *Cicadula divisa,* but only 2 plants developed the symptoms of yellows described in a previous paper.[13] The incubation periods of the disease were 40 and 44 days, respectively, averaging 42 days. Previously noninfective leafhoppers, after feeding on the inoculated plants, failed to transmit the disease to 8 healthy asters and 6 celery plants.

An attempt was made to retain the aster yellows virus through the winter in a perennial plant. Infective leafhoppers were transferred from asters infected with yellows from New York to common plantain or ribgrass (*Plantago major*). Large numbers of insects were reared on common plantain; when one lot of plants became unfavorable as food, the insects were transferred to healthy plants. Common plantain was experimentally infected with yellows and showed typical symptoms of the disease, but the virus was not recovered by previously noninfective leafhoppers during the following spring.

Thamnotettix montanus Van D., a newly discovered vector of California aster and celery yellows, failed to transmit yellows from asters infected in New York to any of 71 healthy asters and 10 celery plants. Previously noninfective *Cicadula divisa* after feeding on the 10 celery plants and some of the asters exposed to *Thamnotettix montanus* failed to transmit yellows to healthy asters and celery.

California aster and celery yellows has been transmitted on rare occasions from a feeding solution containing crushed infective *Cicadula divisa* bred on diseased plantain or ribgrass (*Plantago major*). The feeding solution containing the crushed infective leafhoppers was centrifuged at 3,500 r.p.m. for 1 hour and a portion was fed directly to previously noninfective leafhoppers, while the remainder was filtered through coarse and fine Berkefeld candles and the filtrate was fed to previously

noninfective leafhoppers. The methods of feeding the insects were the same as those used with the beet leafhopper and described in previous papers.[12, 16, 17]

Similar experiments were performed with feeding solutions containing crushed infective *Cicadula divisa* which had fed on yellows-infected asters from New York and Wisconsin. Previously noninfective leafhoppers were fed on the centrifuged feeding solutions containing the crushed infective leafhoppers and also on the filtrates. The feeding solutions contained autoclaved filtered root juice from celery, celeriac, carrot, or parsnip plants, or a combination of petiole and root juice from these plants, various proportions of a 2 per cent solution of maltose, and sometimes a 2 per cent solution of soluble starch solution. The same percentage of maltose, or soluble starch, or a combination of both without the plant extract, was also used. The infective leafhoppers were also crushed in sterile distilled water. All efforts to transmit yellows to 130 healthy asters by feeding previously noninfective leafhoppers on centrifuged feeding solutions or on the filtrates failed.

CARROT-YELLOWS VIRUS FROM MAINE

D. Folsom, of the Maine Agricultural Experiment Station, sent ornamental flowering plants, plantain, and carrots naturally infected with yellows, but only the carrots and a species of *Calendula* arrived in good condition.

Experiments were conducted to determine whether the virus could be transmitted by *Cicadula divisa* from carrots infected with yellows in Maine to healthy asters and celery. Previously noninfective leafhoppers after being exposed to carrots naturally infected with yellows obtained from Maine were transferred to 17 healthy asters and 17 healthy celery plants. One typical case of aster yellows developed and one celery plant showed symptoms of yellows, both being transmitted from the same diseased carrot plant. Previously noninfective leafhoppers failed to transmit yellows from the infected celery to several healthy celery plants.

Previously noninfective leafhoppers, after being exposed to yellows-infected carrot plants received from Maine, transmitted yellows to 3 white, 1 yellow, and 7 orange varieties of carrots. Yellows was not transferred by leafhoppers from the experimentally infected carrots to 11 healthy asters and 11 celery plants, as shown in table 3. The incubation period of the disease in the plants varied from 19 to 81 days, with an average of 48.7 days, as indicated in table 3.

Hollow Crown parsnip (*Pastinaca sativa*) was experimentally infected with yellows by previously noninfective leafhoppers which had

been exposed to naturally infected carrots obtained from Maine. The virus was not recovered from infected parsnips by leafhoppers, for they failed to transmit yellows to healthy asters and celery.

Yellows was transmitted by previously noninfective leafhoppers from naturally infected *Calendula sp.* from Maine to healthy asters but not to celery.

TABLE 3

INCUBATION PERIOD OF MAINE CARROT YELLOWS IN EXPERIMENTALLY INFECTED CARROTS AND RECOVERY OF VIRUS BY *Cicadula divisa*

Variety of carrot	Number of plants inoculated	Number of leaf-hoppers on each plant	Number of plants infected	Number of plants healthy	Incubation period in plant, days	Yellows transferred	
						From carrot to aster	From carrot to celery
Short White	1	55	1	0	54	—	—
White Mastodon	1	50	1	0	54	—	—
White Belgian	1	50	1	0	81	—	—
Yellow Belgian	1	50	1	0	52	—	—
Chantenay	1	50	1	0	45	—	—
Danvers Half Long	1	30	1	0	19	—	—
Early Scarlet Horn	1	50	1	0	54	— ·	—
French Forcing	1	50	1	0	52	—	—
Long Orange	1	30	1	0	44	—	—
Nantes	1	30	1	0	37	—	—
Oxheart or Guerande	1	30	1	0	44	—	—
Total	11	....	11	0	....	11 —	11 —
Average	....	43	....	....	48.7	....	....

ASTER-YELLOWS VIRUS FROM INDIANA

Asters infected with yellows were sent by R. W. Samson, of the Purdue University Agricultural Experiment Station, La Fayette, Indiana.

Transmission experiments were conducted with *Cicadula divisa* to determine whether asters, celery, and parsnips could be infected with yellows from diseased asters received from Indiana. Previously noninfective leafhoppers were exposed for a period of 1 or 2 days on asters infected with yellows from Indiana and were then transferred in lots of 10 or 20 to healthy asters and celery. Ten asters were inoculated with yellows and 5 typical cases of aster yellows developed, while 5 plants failed to show symptoms of the disease. Previously noninfective leafhoppers exposed to the 5 infected asters failed to transmit yellows to 5 healthy celery plants. Ten celery plants exposed to infective leafhoppers failed to develop symptoms of celery yellows.

Hollow Crown parsnip was experimentally infected with aster yellows

from Indiana and showed typical symptoms of the disease as described in a previous paper.[13] The virus was not transferred by leafhoppers from infected parsnips to healthy asters and celery.

Common plantain or ribgrass (*Plantago major*) was experimentally infected with yellows during the autumn and showed typical symptoms of the disease, but the virus was not recovered by previously noninfective leafhoppers during the following spring.

ASTER-YELLOWS VIRUS FROM WISCONSIN

Asters naturally infected with yellows were received from A. J. Riker, of the Wisconsin Agricultural Experiment Station, Madison, Wisconsin.

Asters, celery, and parsnip were inoculated by means of *Cicadula divisa* with the virus of aster yellows obtained from Wisconsin. Twenty-six healthy asters were inoculated, and 18 plants developed typical symptoms of aster yellows. Six of the 82 celery plants inoculated showed symptoms of celery yellows. The virus was transferred by previously noninfective leafhoppers from 2 of the 6 celery-yellows plants to asters.

Hollow Crown parsnip was experimentally infected with aster yellows from Wisconsin, but the virus was not transferred by previously non-infective leafhoppers from infected parsnips to healthy asters and celery.

Three white, 1 yellow, and 7 orange varieties of carrots were experimentally infected with yellows from asters naturally infected in Wisconsin. Nineteen of the 22 inoculated carrots showed typical symptoms of carrot yellows as indicated in table 4. Previously noninfective leaf-hoppers exposed to the inoculated carrots failed to transmit yellows to healthy asters and celery, as shown in table 4. The incubation period of the disease varied from 14 to 44 days, with an average of 29.8 days (table 4).

Male *Thamnotettix montanus* exposed to yellows-infected aster plants from Wisconsin failed to transmit yellows to 18 healthy asters.

CARROT YELLOWS FROM IDAHO

C. F. Henderson,[4] of the United States Department of Agriculture Bureau of Entomology, reported that carrots infected with yellows occurred in Twin Falls, Jerome, and Cassia counties, Idaho, during 1930. He found 17 per cent of the carrots infected with yellows in one field near Twin Falls that year, but during the season of 1932 carrot yellows

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[4] Henderson, C. F., letter to author dated November 29, 1932.

was rarely observed in the vicinity of Twin Falls. Henderson sent several shipments of carrots naturally infected with yellows collected near Twin Falls, and the foliage symptoms were identical with carrot yellows in California.

TABLE 4

INCUBATION PERIOD OF WISCONSIN ASTER YELLOWS IN EXPERIMENTALLY INFECTED CARROTS AND RECOVERY OF VIRUS BY *Cicadula divisa*

Variety of carrot	Number of plants inoculated	Number of leaf-hoppers on each plant	Number of plants infected	Number of plants healthy	Incuba-tion period in plant, days	Yellows transferred	
						From carrot to aster	From carrot to celery
Short White	1	20♂	1	0	29	—	—
	1	20♀	0	1	....	—	—
White Mastodon	1	20♂	1	0	31	—	—
	1	20♀	0	1	....	—	—
White Belgian	1	20♂	1	0	42	—	—
	1	20♀	1	0	23	—	—
Yellow Belgian	1	20♂	1	0	42	—	—
	1	20♀	1	0	28	—	—
Chantenay	1	20♂	1	0	14	—	—
	1	20♀	1	0	28	—	—
Danvers Half Long	1	20♂	1	0	42	—	—
	1	20♀	1	0	28	—	—
Early Scarlet Horn	1	20♂	1	0	22	—	—
	1	20♀	1	0	23	—	—
French Forcing	1	20♂	1	0	18	—	—
	1	20♀	1	0	23	—	—
Long Orange	1	10♂	1	0	30	—	—
	1	10♀	1	0	26	—	—
Nantes	1	20♂	1	0	29	—	—
	1	20♀	1	0	44	—	—
Oxheart or Guerande	1	20♂	1	0	44	—	—
	1	20♀	0	1	....	—	—
Total	22	....	19	3	....	22 —	22 —
Average	....	....	....	....	29.8	....	....

Transmission of yellows by previously noninfective *Cicadula divisa* from naturally infected carrots obtained from Idaho to healthy carrots was accomplished with 3 white, 1 yellow, and 5 orange varieties, as shown in table 5. Previously noninfective leafhoppers exposed to the experimentally infected varieties of carrots failed to transmit yellows to

healthy asters and celery. The incubation period of the disease in the plants varied from 24 to 48 days, with an average of 34.2 days, as indicated in table 5.

In another experiment previously noninfective males reared on barley, which is immune to aster yellows, were exposed to carrots naturally

TABLE 5

INCUBATION PERIOD OF IDAHO ASTER YELLOWS IN EXPERIMENTALLY INFECTED CARROTS AND RECOVERY OF VIRUS BY *Cicadula divisa*

Variety of carrot	Number of plants inoculated	Number of leaf-hoppers on each plant	Number of plants infected	Number of plants healthy	Incubation period in plant, days	Yellows transferred	
						From carrot to aster	From carrot to celery
Short White	1	25♀	1	0	24	—	—
White Mastodon	1	10♀	1	0	45	—	—
	1	30♀	1	0	48	—	—
White Belgian	1	25♀	0	1	....	—	—
	1	10♀	1	0	41	—	—
Yellow Belgian	1	25♀	1	0	31	—	—
Chantenay	1	25♀	1	0	27	—	—
French Forcing	1	10♀	1	0	43	—	—
	1	20♀	1	0	29	—	—
Long Orange	1	25♀	0	1	....	—	—
	1	20♀	1	0	31	—	—
	1	20♀	1	0	29	—	—
	1	20♀	1	0	33	—	—
Nantes	1	25♀	1	0	32	—	—
Oxheart or Guerande	1	25♀	1	0	31	—	—
Total	15	....	13	2	....	15—	15—
Average	...	....	...	....	34.2	....	....

infected with yellows from Idaho and then transferred to healthy asters and celery. In other tests previously noninfective females deposited eggs in the foliage of the diseased carrots, and after nymphs hatched and acquired the winged stage, the males were transferred to healthy asters and celery. Twenty-seven inoculated asters failed to show symptoms of yellows. Sixty-one celery plants were inoculated and 3 of them showed a chlorotic condition of the central leaves with a marked twisting of the petioles. The virus was not recovered from the 3 celery plants showing symptoms of yellows.

CELERY YELLOWS FROM UTAH

In 1927 Linford[7] reported a yellows disease on celery in Salt Lake and Weber counties, Utah. He first observed aster yellows on September 9, 1927, in four localities in Salt Lake and Davis counties, Utah, with a maximum severity of 3 per cent.

Kunkel[6] states, however, that there is no convincing evidence that celery yellows reported in Utah is California yellows.

H. L. Blood, United States Department of Agriculture Bureau of Plant Industry, stationed at the Utah Agricultural Experiment Station, sent 3 small celery-yellows plants from Salt Lake City. Previously noninfective *Cicadula divisa*, after feeding on the celery-yellows plants, transmitted yellows from 2 of the 3 plants to 2 healthy celery plants and 1 aster plant. Unfortunately one of the inoculated aster plants died before symptoms of aster yellows developed. The virus was transferred by previously noninfective *Cicadula divisa* from the 2 experimentally infected celery plants to 2 healthy celery plants. Celery yellows of Utah is probably identical with California aster yellows.

YELLOWS AND CURLY TOP OF ZINNIA

Severin[10] reported that a circular bed of zinnias (*Zinnia elegans*) showing 100 per cent California yellows was found in the center of a lawn in front of the Spreckels Agricultural Experiment Station. *Cicadula divisa* was abundant on the zinnias and on the grass. The plants were stunted, chlorotic, and with abnormal flowers. Noninfective leafhoppers after feeding on the diseased zinnias transmitted yellows to asters and celery.

Kunkel[6] failed to infect *Zinnia elegans* with California yellows experimentally. He was able to infect *Zinnia multiflora* L. with the New York aster-yellows virus, but this species is not grown for seed production in California.

During the summer of 1932, several surveys were made of the yellows and curly-top diseases of ornamental flowering plants grown on seed farms in the San Juan and Salinas valleys. Different varieties and hybrids of *Zinnia elegans* on both seed farms were stunted and showed a yellowing of the apical and secondary shoots. Three varieties of *Zinnia elegans* commonly known as Double Giant Pink, Dahlia Flowered mixed, and Lilliput Scarlet Gem were demonstrated to be naturally infected with yellows. Previously noninfective *Cicadula divisa* after feeding on the 3 varieties of *Zinnia elegans* transmitted yellows to healthy celery.
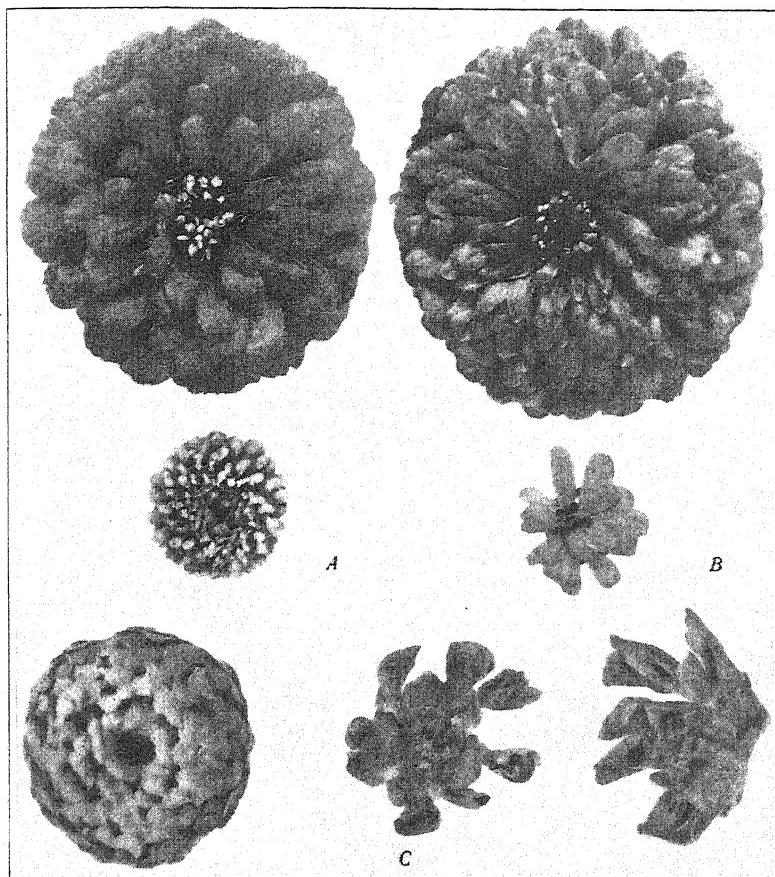
Fig. 1.—*A*, Giant Red zinnia (*Zinnia elegans*): upper, flower from check or control plant; lower, dwarfed flower which was green instead of red in color, from a plant experimentally infected with California aster yellows. *B*, Dahlia Flowered Orange zinnia: upper, flower from check or control plant; lower, dwarfed flower from a plant infected with curly top showing few petals, which were normal in color. *C*, Other abnormal flowers, which were green in color instead of red, from Giant Red zinnia, experimentally infected with California aster yellows.

Previously noninfective beet leafhoppers, *Eutettix tenellus*, after feeding on 9 diseased *Zinnia elegans* transmitted curly top to healthy sugar beets. Four of the 9 zinnias are commonly known as Double Giant type brightness, and were grown adjacent to garden, table, or red beets in the San Juan Valley.

During the summer of 1933 no zinnias infected with yellows or curly top were found on the same seed farms. In the Salinas Valley 40 per cent of the asters were naturally infected with yellows in some plots. Varieties

of zinnias were completely surrounded with diseased asters, but no zinnia yellows was found.

It was decided to attempt experimental infection of different species, varieties, and hybrids of zinnias with California yellows and curly top. Twenty-six Mexican Double Orange zinnias (*Zinnia haageana*) were
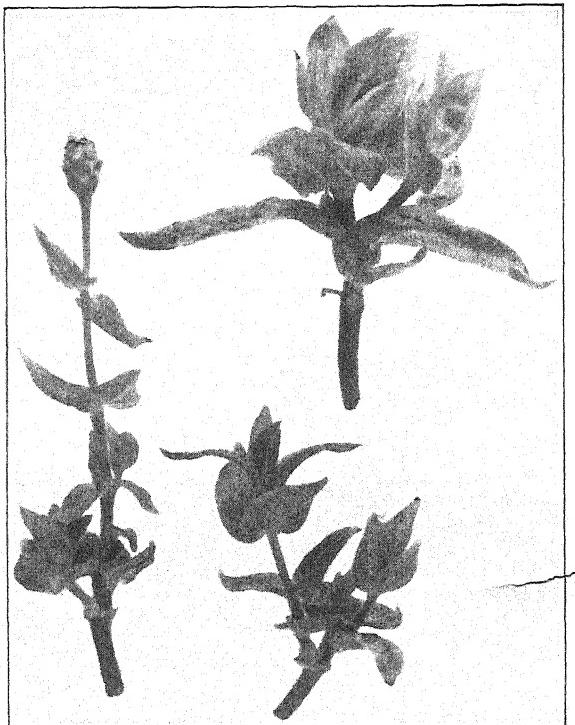
Fig. 2.—Giant White zinnia (*Zinnia elegans*) experimentally infected with curly top showing secondary shoots and inward-cupped leaves.

repeatedly inoculated with yellows by lots of 5 or 10 infective *Cicadula divisa*. Three plants developed symptoms of yellows with chlorotic secondary shoots and dwarfed yellow flowers, but the flowers were not abnormal in color. Previously noninfective leafhoppers recovered the virus from the infected plants and transmitted it to healthy asters and celery. No difficulty was experienced in experimentally infecting this species of zinnia with curly top.

Twenty-five varieties or hybrids of *Zinnia elegans* were each inoculated by 2 lots of 10 infective *Cicadula divisa*. Double Pompom White Gem and Giant Red zinnias developed symptoms of yellows, but the virus

was not recovered by previously noninfective leafhoppers. The flowers failed to expand (fig. 1 *A, C*) and were green in color.

The following varieties or hybrids of *Zinnia elegans* were experimentally infected with curly top and the virus was recovered by previously noninfective beet leafhoppers and transferred to sugar beets: Dahlia Flowered Lavender, Dahlia Flowered Orange (fig. 1 *B*) Dahlia Flowered Red, Dahlia Flowered Rose, Double Dahlia Flowered Golden Yellow, Double Dahlia Flowered Light Yellow, Double Dahlia Flowered White, Double Elegans Golden Yellow, Double Elegans Bright Scarlet, Double Elegans Salmon Rose, Double Giant Canary Yellow, Double Lilliput (Dwarf Miniature), Giant Orange, Giant Pink, Giant Purple, Giant Red, Giant White (fig. 2), Double Pompom Dark Crimson, Double Pompom Golden Gem, Double Pompom Salmon Rose, Double Pompom White Gem, Lilliput Crimson Gem, Lilliput Golden Gem, Lilliput Salmon Rose, and Lilliput Pompom Scarlet Gem.[5]

The symptoms of zinnia curly top could not be distinguished from zinnia yellows on old plants in the field, but no study has been made of the symptoms of the two diseases on young plants. Young plants experimentally infected with curly top showed cleared or transparent veinlets, but on old plants infected under natural conditions, this symptom could not be distinguished from normal venation. The internodes near the apices of the branches were shortened with chlorotic secondary shoots arising from the axil of the leaves (fig. 2). The leaves frequently were cupped inward along the midrib. The flowers were dwarfed, with the petals reduced in number but not abnormal in color (fig. 1 *B*).

DISCUSSION

Recovery of Virus.—Kunkel experienced difficulty in the recovery of the aster-yellows virus in New York from experimentally infected host plants. In his first paper Kunkel[4] lists 64 species of plants in 23 families that were experimentally infected with aster yellows, but the virus was transferred back to asters from only 32 species. In a later paper Kunkel[5] reported transmitting yellows to 120 species of plants in 30 families, but the virus was recovered from only 12 species. He states, however, in his second paper that ,"while such transmission is necessary in order to bring full proof that the disease observed on any yellowed plant is actually aster yellows, the symptoms are so similar on different hosts that this step was not considered necessary in most cases."

[5] The first seventeen varieties or hybrids were grown from seeds obtained from the Ferry-Morse Seed Co. (1932 catalog) San Francisco, California; the next four from the Germain Seed & Plant Co. (1932 catalog) Los Angeles, California; and the last four from Peter Henderson & Co. (1932 catalog) 35 Cortland St., New York.

The recovery of the aster-yellows virus obtained from other states from host plants showing symptoms of the disease is given in the summary of this paper.

It was sometimes impossible to recover the California aster-yellows virus from naturally infected weeds and experimentally infected economic cultivated plants showing typical symptoms of the disease. Cross inoculations from experimentally infected Double-Curled, Extra-Triple

Fig. 3.—Common buckwheat (*Fagopyrum esculentum*): left, shoot from a plant experimentally infected with California yellows showing large number of flowers with petals which were green in color; right, shoot from a healthy plant used as a check or control showing white flowers.

Curled, and Fern-Leaf or Moss-Curled parsley showing symptoms of yellows were failures, as reported in a previous paper.[13]

Host-Range Differences.—There is some evidence to show that host-range differences occur with California and New York aster yellows. Numerous attempts were made by Kunkel[5] to transmit the aster-yellows virus of New York to potatoes by means of the insect vector. The varieties used included Irish Cobbler, Green Mountain, Bliss Triumph, and Spaulding Rose.

During the past five years, California yellows was transmitted to a number of varieties of potatoes including Bliss Triumph.[14] It was impossible, however, to recover the virus from experimentally infected varieties of potatoes showing symptoms of the disease.

Overlapping Host Ranges.—The overlapping economic host plants of California aster yellows and aster yellows of New York so far investigated are as follows: common buckwheat (*Fagopyrum esculentum*) (fig. 3), spinach (*Spinacia oleracea*), carrot (*Daucus carota* var. *sativa*), dill (*Anethum graveolens*), parsnip (*Pastunaca sativa*), peasant's tobacco (*Nicotiana rustica*) (fig. 4), salsify (*Tragopogon porrifolius*), and lettuce (*Lactuca sativa*). The symptoms of the disease on these over-

Fig. 4.—Peasant's tobacco (*Nicotiana rustica*): *A*, cluster of secondary shoots from a plant experimentally infected with California yellows; *B*, apical shoot from a check or control plant.

lapping host plants infected with the California and New York aster yellows appear to be identical.

It is not to be expected that two different viruses would have identical host ranges. It is not uncommon for two different virus diseases to have overlapping host ranges or to produce similar symptoms.[5] It is difficult to explain, however, why an occasional celery plant developed symptoms of yellows with the aster-yellows virus from other states and the leafhoppers were not able to recover the virus except on very rare occasions. Similar difficulties were encountered with resistant host plants of curly top such as pink beans (*Phaseolus vulgaris*) and Australian saltbush (*Atriplex semibaccata*), as reported in a previous paper.[15]

Smith[18] expressed the opinion that slight differences in the host range do not justify the separation into distinct viruses of entities which are otherwise identical.

Strains of Aster Yellows.—Strains or variants of the aster-yellows viruses transmitted by different species of leafhoppers may occur in the United States, Bermuda Islands,[9] Japan,[8] or Europe.

According to Kunkel,[6] "whether the yellows from California is a strain of aster yellows or is a different disease is a question that cannot be answered at this time." The facts that both are transmitted by the same insect vector, have long incubation periods in the leafhopper, and produce similar symtoms in aster and some other host plants, support the view that they may be related.

According to Smith,[18] "Perhaps the best illustration of two apparently independent strains of a plant virus is afforded by the case of aster yellows and celery yellows. .... Here then is apparently a case of a virus having 'mutated' or adapted itself to a new host plant in one district and after sojourn in this host [having] acquired the ability to infect it as easily as any other plant in its host range. Such a virus may be regarded merely as a slightly different strain of aster yellows, or it may be regarded as a different entity and be referred to as 'celery yellows.' It is also possible that celery yellows is a stage in the evolution of an entirely new virus."

SUMMARY

Yellows was transmitted by previously noninfective *Cicadula divisa* from asters naturally infected in New York, Indiana, and Wisconsin to asters. Previously noninfective leafhoppers exposed to asters or salsify infected with the disease in New York transmitted yellows to 8 of the 207 celery plants inoculated. The virus was transferred from 1 experimentally infected celery plant to 3 successive healthy asters, but was not transferred from the 3 infected asters back to 12 healthy celery plants. The virus from yellows-infected asters in Wisconsin was transferred to 6 of the 82 celery plants inoculated and from 2 of the 6 experimentally infected celery plants back to asters. Ten celery plants inoculated with the virus of aster yellows from Indiana failed to develop symptoms of the disease.

Yellows was transmitted from celery naturally infected with yellows in Utah to aster and celery plants. The virus was recovered from the experimentally infected celery plants and again transferred to healthy celery plants.

Yellows was readily transmitted to healthy carrots from asters naturally infected with the disease in New York, Maine, and Wisconsin. The transfer of yellows by previously noninfective leafhoppers from experimentally infected carrots to healthy asters was accomplished with 6 of the 22 plants with the aster-yellows virus obtained from New York, but was not performed with 22 healthy celery plants. All efforts to transfer yellows from experimentally infected carrots to healthy asters or celery

with the virus of aster yellows obtained from Maine and Wisconsin failed. No difficulty was experienced in transmitting aster yellows to healthy carrots from carrots naturally infected with the disease in Maine and Idaho. Yellows was transferred from one carrot naturally infected with the disease in Maine to aster and celery, but the virus was not transferred from the infected aster to celery nor from the celery-yellowed plant to any of several healthy celery plants. The transfer of yellows from carrots naturally infected with the disease in Idaho was accomplished with 3 of the 61 celery plants inoculated, but the virus was not recovered from the 3 celery plants showing symptoms of yellows.

Single or Plain parsley, Hamburg or Turnip-rooted parsley, and common plantain or ribgrass (*Plantago major*) were experimentally infected with yellows by previously noninfective leafhoppers which had been exposed to aster yellows obtained from New York, but the virus was not recovered from any of the infected plants. Hollow Crown parsnip was experimentally infected with yellows with the aster-yellows virus from Indiana and Wisconsin and carrot-yellows virus from Maine, but the virus was not recovered from the infected parsnips. The number of tests, however, with all of the species or varieties of plants, was not sufficient to state that the virus could not be recovered on rare occasions.

Thamnotettix montanus Van D., a newly discovered vector of California aster and celery yellows, failed to transmit yellows from asters naturally infected with the disease in New York and Wisconsin to healthy asters and celery.

The results of this investigation show that carrots and asters can be experimentally infected with the aster-yellows virus obtained from New York, Indiana, and Wisconsin, also the carrot-yellows virus from Maine and Idaho, and with the aster-yellows virus from California. Celery was found to be highly resistant to the aster or carrot-yellows virus obtained from all states except California.[10]

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TRANSMISSION OF CALIFORNIA ASTER YELLOWS TO POTATO BY CICADULA DIVISA

HENRY H. P. SEVERIN AND FRANK A. HAASIS

TRANSMISSION OF CALIFORNIA ASTER YELLOWS TO POTATO BY CICADULA DIVISA[1]

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(Contribution from the Division of Entomology and Parasitology, California Agricultural Experiment Station, University of California, coöperating with the United States Department of Agriculture Bureau of Entomology.)

INTRODUCTION

KUNKEL[(1)] FAILED TO TRANSMIT New York aster yellows to potato (*Solanum tuberosum*) by means of the insect vector, *Cicadula divisa* Uhl. [*C. sexnotata* (Fall.)]. The following varieties of potatoes were either immune or highly resistant to the aster-yellows disease: Irish Cobbler, Green Mountain, Bliss Triumph, and Spaulding Rose.

An investigation was undertaken to determine whether potato plants could be experimentally infected with California aster yellows. A study of the symptoms and incubation period of the disease in the plant was made. Attempts were made to recover the virus from infected plants by means of previously noninfective leafhoppers. Trips were made to the potato fields in the delta districts of the San Joaquin Valley to determine whether this virus disease occurs under natural conditions, and observations were made on the relative abundance of the leafhopper on potato plants during the season.

METHODS

The varieties of potatoes used were Bliss Triumph, White Rose, and potatoes grown from seeds. The potatoes were grown in 12-inch flower pots or in large wooden pickle tubs filled with peat soil. The potato plants were enclosed in large cages and inoculated with yellows by 20 to 40 infective leafhoppers. Males were used rather than females so as to avoid egg deposition. The insects inoculated the plants during a period of 1 to 10 days and then the cages containing the males were removed from the plants. The inoculated plants were fumigated with Nico-Fume tobacco-paper insecticide after inoculation and were kept in

a greenhouse free from leafhoppers or out-of-doors in insect-proof
cages. Check or control plants grown from the same tubers were used.
Noninfective leafhoppers were kept on some of the control plants, while
others were kept free from insects.

RESULTS

During a period of five years, 104 potato plants were inoculated with the
California aster-yellows virus by means of infective *Cicadula divisa,*
and 50 per cent of the inoculated plants developed symptoms of the dis-
ease, as is shown in table 1.

TABLE 1

RESULTS OF INOCULATION OF POTATO PLANTS WITH YELLOWS VIRUS BY
CICADULA DIVISA

Dates leafhoppers inoculated plants	Number of potato plants inoculated	Number of leafhoppers on each plant	Number of plants infected	Number of plants healthy	Per cent of plants infected
Sept. 12–15	14	25	14	0	100.0
Sept. 18–23	13	20	13	0	100.0
Oct. 8– 9	12	30	7	5	59.3
Oct. 9–11	11	30	1	10	9.0
Oct. 16–17	8	25	4	4	50.0
Oct. 17–18	8	25	0	8	0.0
Oct. 30–Nov. 2	5	25	2	3	40.0
Feb. 1– 4	1	40	0	1	0.0
Feb. 11–21	2	40	0	2	0.0
Feb. 21–28	1	40	1	0	100.0
Mar. 10–19	1	40	0	1	0.0
Apr. 12–May 9*	1	20	0	1	0.0
Apr. 15–May 9*	2	20	0	2	0.0
Apr. 18–May 9*	2	20	0	2	0.0
Apr. 26–May 13*	4	20	2	2	50.0
June 5–18	4	25	2	2	50.0
June 9–18	5	25	3	2	60.0
June 11–18*	4	35	1	3	25.0
June 11–18	5	35	1	4	20.0
July 7 –18	1	25	1	0	100.0
Total or percentage	104	....	52	52	50.0

* Potato plants grown from seeds.

Symptoms.—The most pronounced symptoms which appeared on po-
tato plants infected with the California aster-yellows virus were purple
slender sprouts (figs. 1*A*, 2*A*, *B*, 3 *C–M*) and purple sessile aerial tubers[4]
(figs. 1*B*, 4) which developed from the axils of the leaves. Sometimes
aerial tubers developed at the end of the sprouts (figs. 1*A*, 3*B*). Fre-

[4] Richard and Blood[2] described and figured aerial tubers in their contribution on
psyllid yellows of the potato. H. L. Blood, E. S. Schultz, and M. Shapovalov have
examined potato plants showing symptoms of California aster yellows, and all agreed
that the symptoms were not identical with those of psyllid yellows.
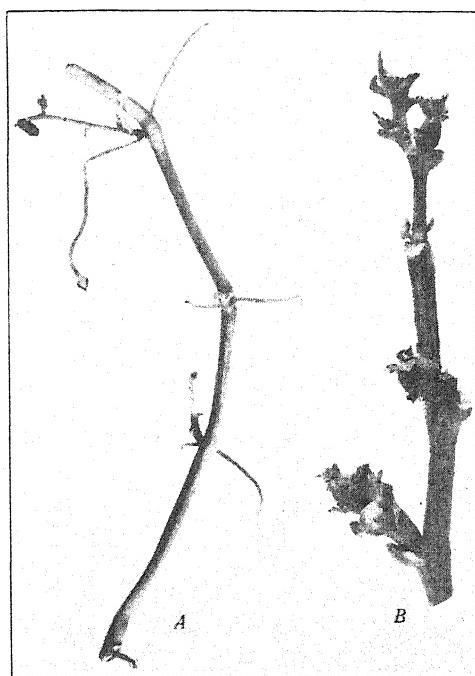
Fig. 1.—Stems of potato plant infected with yellows with leaves removed: *A*, slender sprouts; *B*, sessile aerial tubers growing from buds at the nodes.
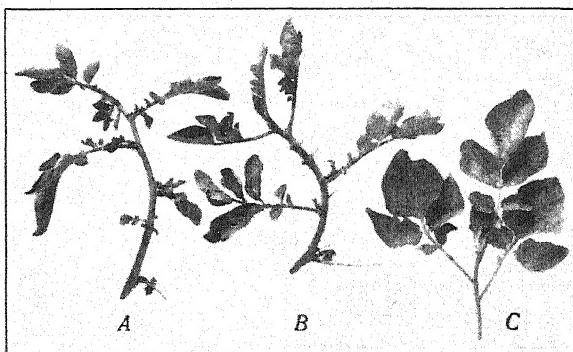
Fig. 2.—*A*, *B*, Shoots from potato plant infected with yellows showing slender sprouts growing from the axils of the leaves; *C*, shoot from check or control plant from the same tuber.
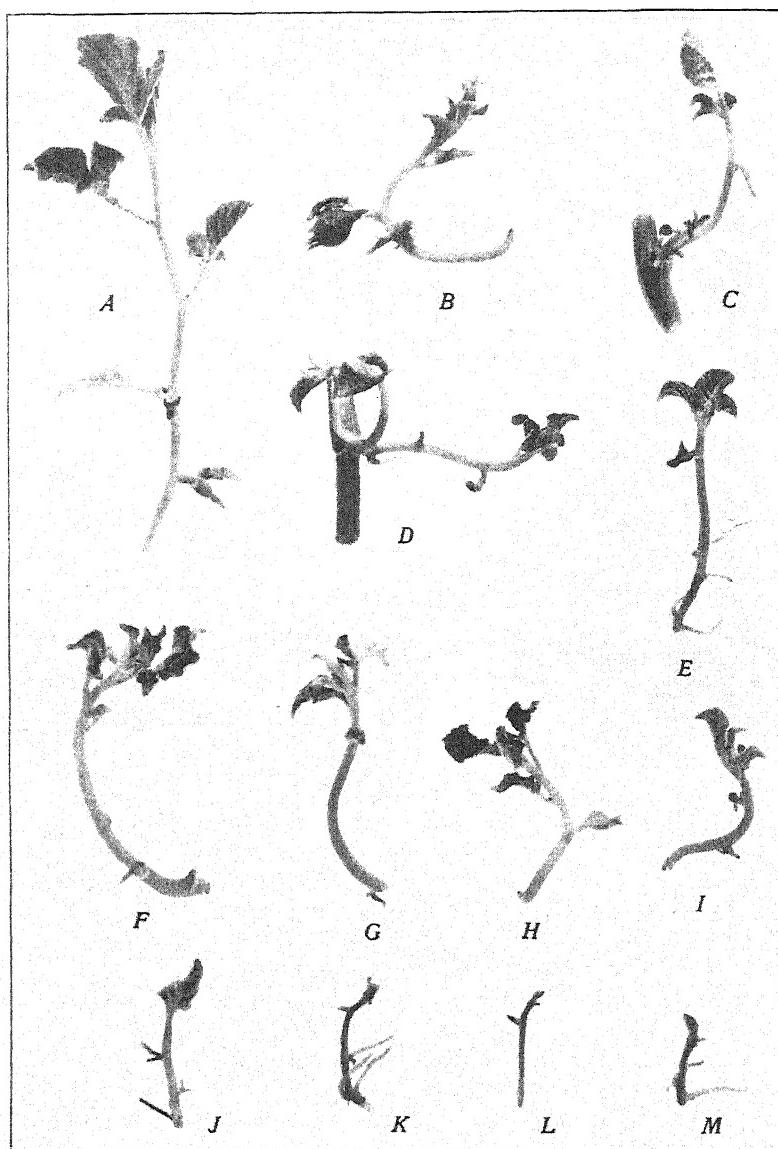
Fig. 3.—Axillary shoots from potato plants grown from seeds infected with yellows: *A*, normal leaves; *B*, aerial tuber; *C–I*, curved petioles with terminal leaflets and lateral sprouts; *J–M*, petioles with lateral sprouts which may represent the veins of undeveloped leaves.

quently dwarfed leaves developed on the aerial tubers (fig. 1*B*). The margins of the leaves were rolled inward (figs. 2*A*, *B*) with the petioles often bent or curved downward (fig. 4). The leaves and stems were brittle. In the later stages of the disease the lower leaves turned yellow and became dry (fig. 4).
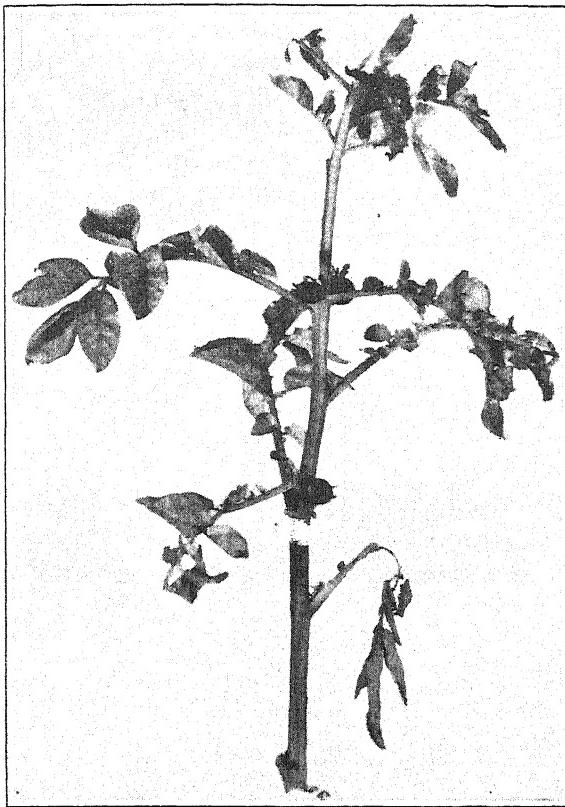
Fig. 4.—Shoot from potato plant infected with yellows showing sessile tubers growing from the axils of the leaves, and dried lower leaf.

Potato plants grown from seeds were infected with yellows and the symptoms of the disease were studied. The internodes were shortened and secondary shoots with or without leaves developed from the axils of the leaves (fig. 3*B–J*). The secondary shoots frequently developed purple, slender, lateral sprouts, which may represent veins of leaflets which failed to develop (fig. 3*C–M*) as in the case of shoe-string mosaic of tomatoes. Purple aerial tubers sometimes grew on the secondary shoots (fig. 3*B*).

Incubation Period of Disease in the Plant.—The length of time that elapsed from the inoculation of the potato plants until slender sprouts or aerial tubers developed in the axils of the leaves was considered as the incubation period of the disease and varied as follows: autumn 20 to 37 days; late winter and early spring 50 to 63 days; and summer 27 to 40 days.

Recovery of Virus.—All attempts to recover the virus from potato plants which developed symptoms of yellows disease were failures. Noninfective leafhoppers were fed on all of the infected potato plants and were transferred to healthy asters and celery but not a single case of aster or celery yellows developed. Noninfective leafhoppers failed to recover the virus by feeding on the cut surfaces of potato tubers obtained from plants which had shown symptoms of the yellows. No experiments have been conducted up to the present time on transmitting yellows from infected to healthy potato plants by grafting or budding.

Controls.—Plants grown from cuttings of each tuber and potato plants grown from seeds were used as checks or controls. Noninfective leafhoppers were fed on some controls, others were kept free from insects. All controls remained healthy.

Under Natural Conditions.—Potato plants infected with yellows have not been found under natural conditions up to the present time. *Cicadula divisa*, however, was taken in small numbers in the potato fields in the delta districts of the San Joaquin Valley, throughout the growing season in 1930–1932, but the beet leafhopper, *Eutettix tenellus* (Baker) was more abundant, especially during 1932.

SUMMARY

Fifty per cent of the potato plants inoculated with California aster yellows developed symptoms of the disease. The most pronounced symptoms of the disease were purple slender sprouts and aerial tubers arising from the axil of the leaves. The incubation period of the disease varied from 20 to 63 days during the four seasons. The virus was not recovered from infected potato plants nor from potato tubers obtained from plants showing symptoms of yellows. The disease has not been found in potato fields under natural conditions up to the present time, but *Cicadula divisa* was taken in potato fields.

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TRANSMISSION OF CALIFORNIA ASTER AND CELERY-YELLOWS VIRUS BY THREE SPECIES OF LEAFHOPPERS

HENRY H. P. SEVERIN

TRANSMISSION OF CALIFORNIA ASTER AND CELERY-YELLOWS VIRUS BY THREE SPECIES OF LEAFHOPPERS[1]

HENRY H. P. SEVERIN[2]

(Contribution from the Division of Entomology and Parasitology, California Agricultural Experiment Station, University of California, coöperating with the United States Department of Agriculture, Bureau of Entomology.)

INTRODUCTION

IT HAS BEEN SUGGESTED that possibly an obligate relation exists between a specific insect vector and the aster-yellows virus, and that developmental changes and multiplication of the virus take place during the incubation period in the insect.[8] It has been assumed in the past that the aster-yellows virus could be disseminated only by the leafhopper, *Cicadula divisa* Uhl., which is widely distributed in America.

Ogilvie[10] reported yellows of China aster (*Callistephus chinensis*) in Bermuda, where *Cicadula sexnotata* (Fall.), responsible for the transmission of the virus there, has been known to occur since 1924. The disease also occurs on cos lettuce, cabbage lettuce, eight species of ornamental flowering plants, and several wild plants in Bermuda.

Fukuski[5] reported that aster yellows occurs in Japan. Kunkel[8] reported that *Cicadula sexnotata* occurs in Japan and probably throughout the Orient.

Dobroscky[3] reported that aster yellows was found in the gardens of the Budapest Experiment Station, and in the vicinity of Lake Balaton Biological Laboratory, Hungary. *Cicadula sexnotata* is widespread and common in Europe.[8]

In California three species of leafhoppers transmit the aster-yellows virus. *Cicadula divisa* transmits the virus with greater efficiency than the mountain leafhopper, *Thamnotettix montanus* Van D. or the geminate leafhopper, *T. geminatus* Van D. Experiments with the leafhoppers *Agallia californicum* (Baker), *A. cinera* (O. & B.), and *Empoasca abrupta* De L. bred on celery failed to transmit the yellows virus.[13]

An investigation was undertaken to determine whether or not California aster and celery yellows are caused by two viruses or a single

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[1] Received for publication March 15, 1934.
[2] Associate Entomologist in the Experiment Station.
[3] I. D. Dobroscky, in a personal interview with the author.

virus, and whether the viruses could be separated by the three vectors. Transmission experiments with yellows by the three species of leafhoppers were conducted with the virus obtained from naturally infected asters, celery, and carrots, and from these same host plants experimentally infected by the different species of leafhoppers. Attempts were made to recover the virus from these experimentally infected host plants with the three species of previously noninfective leafhoppers. The host range of the disease among economic plants and weeds was also investigated. The characteristics, distribution, and food plants of two newly discovered vectors of California yellows are discussed in this paper.

METHODS

One method used in the separation of a mixture of viruses in the living plant is by the selective transmission of one virus by the insect vector. Hoggan[7] has shown that the peach aphid, *Myzus persicae* Sulz., transmits only the cucumber mosaic virus from a combination of cucumber and tobacco mosaic viruses, although the tobacco virus was present in the leaves upon which the aphids had fed. Bennett[3] demonstrated that *Aphis rubiphila* Patch transmitted only the curl virus of raspberries and *Amphorophora rubi* Kalt transmitted only the yellow mosaic virus and a medium type of mosaic from a raspberry infected with the three diseases. Smith[16] utilized the peach aphid, *Myzus persicae,* in the separation of potato viruses.

The method adopted to determine whether California aster yellows and celery yellows are caused by two viruses or a single virus was to conduct transmission experiments with yellows by the three species of leafhoppers from naturally and experimentally infected asters, celery, and carrots to healthy plants grown from seeds. It was found that *Cicadula divisa* occurred on all of these host plants in the field. *Thamnotettix montanus* was rare on asters, abundant on celery, and common on carrots. *T. geminatus* was often collected on asters, rarely on celery, and commonly on carrots. These three host plants were transplanted in pots in the greenhouse and healthy plants grown from seeds were used as checks or controls.

Production of Noninfective Leafhoppers.—The production of noninfective *Cicadula divisa* on Sacramento barley immune to the yellows disease has been described in a previous paper.[13] The production of noninfective *Thamnotettix montanus* and *T. geminatus* on barley failed because these species of leafhoppers did not complete their life cycles on that plant. The method adopted was similar to that used with the beet leafhopper, *Eutettix tenellus* (Baker), described in a previous paper.[12]

Several hundred females of each species oviposited in large celery plants for a period of one week and were then removed. Nymphs were transferred after emergence from the egg, before they had an opportunity to feed, from diseased to healthy celery plants, with a fine camel's-hair brush moistened at the tip. The leafhoppers reared to the adult stage on healthy celery, as well as later generations so bred, failed to produce the disease.

DETERMINATION OF THE SPECIES

Thamnotettix Montanus.—The mountain leafhopper, *Thamnotettix montanus*, is 4.5 to 6.0 mm long, with white or yellowish face, a transverse brownish band between the eyes, a conspicuous yellow transverse band on the pronotum, and the scutellum brown (pl. 1, *C, D, E, F*). The summer adults collected in the northern San Joaquin, southern Sacramento, Santa Clara, and Salinas valleys were dark brown (pl. 1, *C, D*) while the specimens taken during the autumn were usually black (pl. 1, *E, F*), with intermediates between the two color patterns.

Thamnotettix montanus has been recorded from British Columbia,[19] Washington,[22, 23] Oregon,[22] California,[20, 22] Nevada,[4] Idaho,[4] and Colorado[6, 19] and probably occurs in most of the western states.

Essig[4] reported that *Thamnotettix montanus* is common on grasses, weeds, carrot, larkspur, goldenrod, apple, and prune.

This leafhopper has a wide range of food plants in California. Adults were collected abundantly on celery growing in the Sacramento Valley, but not so abundantly in the fog belt of the Santa Clara and Salinas valleys. Adults were commonly taken and an occasional nymph on White Icicle radish and Purple Top White Globe turnip in the delta districts near Stockton, in the San Joaquin Valley. The leafhopper was rarely taken on asters in the Salinas Valley. The insects were occasionally captured on the following economic plants in the Sacramento and San Joaquin valleys:

> Chenopodiaceae: sugar beets, garden, red, or table beets.
> Leguminosae: alfalfa and beans.
> Cucurbitaceae: squash, pumpkin, and cucumbers.
> Solanaceae: potatoes.
> Cruciferae: Chinese cabbage.
> Umbelliferae: carrots.
> Compositae: lettuce.

Weeds as food and breeding plants of *Thamnotettix montanus* have received little attention up to the present time. The adults were taken

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[4] Several shipments of *Thamnotettix montanus* were received from Twin Falls, Idaho, collected by C. F. Henderson.

in small numbers on tumbleweed (*Amaranthus graecizans*). Nymphs hatched from eggs deposited in curly dock (*Rumex crispus*) under natural conditions and were reared to the adult stage on this host plant.

Thamnotettix Reductus.—Van Duzee[5] determined the species from California and Idaho as *Thamnotettix reductus*, and "considers *reductus* as a species distinct from *montanus*, although it was described as a variety."[22] De Long[6] could find no genital character which is constant and distinctive on the adults from California and Idaho, but in certain groups it is very difficult to find characters on the genitalia. He states, "I do not feel, however, that *reductus* is a distinct species."

Since *Thamnotettix montanus* and *T. reductus* are considered distinct species on the basis of color pattern only and since there is a difference of opinion among systematists as to the species, the name used in this paper is *montanus*. The description of *T. montanus reductus* by Van Duzee[22] follows:

This form seems to be purely a color variety in which the yellow saddle is reduced to a small mark on the apex of the claval nervures, often on the outer nervures only, or in a few dark males it may be entirely wanting. The brown band on the base of the vertex is also reduced, sometimes to a mere shade, but there may be a dark line next the eye and a geminate spot on the basal middle. Both forms are found together throughout their range, but the present form is much more abundant toward the south, while those from Oregon and Washington are almost entirely typical *montanus*.

Specimens of *Thamnotettix* received from C. F. Henderson, Twin Falls, Idaho, were determined as *T. reductus* by Van Duzee and as *T. montanus* by De Long. The leafhoppers from Idaho transmitted yellows to healthy celery but not to asters. The virus was recovered and transferred by previously noninfective *Cicadula divisa* from the celery infected with yellows by the *Thamnotettix* from Idaho to healthy asters and celery.

Thamnotettix Geminatus.—The geminate leafhopper, *Thamnotettix geminatus*, is 5 to 6 mm long, greenish yellow or brown, with a pair of black spots on the anterior border of the head, a black spot on each side of the eye, an arched band near the front border of the pronotum, and black spots on the scutellum (pl. 1, *G, H*). A more detailed description of the species is given by Van Duzee.[19]

Thamnotettix geminatus has been recorded from Colorado,[6, 18] Idaho,[7] California,[20, 22, 23] Washington,[11] and Alaska.[1] It has been recorded under the name *Cicadula laeta* from Alaska and Shumagin and

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[5] Van Duzee, E. P., letter to author dated November 25, 1930.

[6] DeLong, D. W., letter to author dated August 15, 1932.

[7] Several shipments of *Thamnotettix geminatus* were received from Twin Falls, Idaho, collected by C. F. Henderson.

Popof islands by Ashmead.[1] One specimen under the same name also is in the United States National Museum from Unga Island.[1]

Osborn[11] reported that *Thamnotettix geminatus* occurred in such numbers upon clover, alfalfa, and timothy in the state of Washington, especially at Pullman, as to threaten to become destructive. Additional food plants recorded by Essig[4] include grasses, grains, and apple. Van Duzee[20] found the leafhopper common on *Malvastrum* in San Diego County, California

No intensive study has been made of the food and breeding plants of *Thamnotettix geminatus* in California. The adults were commonly taken on carrots in the Sacramento and Salinas valleys, but rarely on celery, and often on asters in the Salinas Valley.

TRANSMISSION OF YELLOWS BY THAMNOTETTIX MONTANUS TO HEALTHY ASTERS AND CELERY

Collected on Celery Under Natural Conditions.—During 1931 a serious outbreak of celery yellows occurred in the Sacramento and Santa Clara valleys, and celery in many fields was plowed under. *Thamnotettix montanus* was very abundant in the celery fields near Sacramento. Adults captured in the celery fields transmitted yellows to 2 of 12 healthy celery plants but not to asters, as shown in table 3 (p. 348). These results demonstrate that this insect is a vector of celery yellows under natural conditions.

Fed on Naturally Infected Asters and Celery.—A comparison was made of the transmission of yellows by previously noninfective *Cicadula divisa* and *Thamnotettix montanus* from 10 asters naturally infected with the disease to healthy asters and celery. Ten lots, each consisting of 10 *C. divisa* or 10 *T. montanus* were fed for a period of 2 days on 10 diseased asters, one lot to a plant, and then each lot was fed for a period of 21 days on a healthy aster or celery plant; *T. montanus* was used only on celery. Each lot was then transferred to successive healthy aster or celery plants and was kept on each plant for a period of 10 days. In the recovery of the virus from celery experimentally infected by *T. montanus* with the virus from naturally infected asters, the feeding period on the infected celery plants varied from 4 to 33 days. The results obtained are indicated in table 1.

The results in table 1 show that previously noninfective *Cicadula divisa* after feeding 2 days on asters naturally infected with yellows transferred the virus to 45 per cent of the healthy asters and to 48.3 per cent of the healthy celery plants. Previously noninfective *Thamnotettix mon-*

tanus after feeding 2 days on asters naturally infected with yellows transferred the virus to 20 per cent of the healthy celery plants as shown in table 3, and recovered the virus from 3 of the 4 celery plants which they had infected (table 1), or 75 per cent.

Tests were made on the transmission of yellows by *Thamnotettix montanus* from naturally infected celery. Seventeen lots of 10 or 20 *T. mon-*

TABLE 1

Comparison of Transmission of Yellows by Previously Noninfective Cicadula Divisa and Thamnotettix Montanus from Naturally Infected Asters to Successive Healthy Asters and Celery, and Recovery of Virus by T. Montanus*

Source of inoculation: aster-yellows plant no.	Successive aster and celery plants inoculated							Virus recovered from celery infected by *T. montanus* and transferred by this leafhopper to celery	
	By *C. divisa*					By *T. montanus*			
	Asters		Celery			Celery			
	First set	Second set	First set	Second set	Third set	First set	Second set	First set	Second set
1	+	+	+	+	+	−	−	....	....
2	+	+	+	+	....	−	+	+	−
3	−	−	−	−	−	−	−	....	....
4	−	−	−	−	−	−	−	....	....
5	−	+	+	+	−	−	+	±	+
6	−	−	−	−	−	−	−	....	....
7	−	−	−	−	−	−	−	...	....
8	+	+	+	−	−	−	+	−	−
9	+	+	+	+	+	−	+	−	+
10	−	−	+	+	+	−	−	....	....
Total +	4+	5+	6+	5+	3+	0+	4+	1+	2+
Total −	6−	5−	4−	5−	6−	10−	6−	3−	2−

* The plus sign (+) indicates the production of the disease, and the minus sign (−) shows that no disease resulted.

tanus each were fed for a period of 3 weeks on diseased celery plants. Each lot was then transferred at weekly intervals to 3 successive healthy celery plants and to 1 aster. A comparison was also made of the recovery of the virus by previously noninfective *T. montanus* and *Cicadula divisa* from celery experimentally infected with yellows by *T. montanus*. The same procedure was used in the recovery of the virus from experimentally infected celery with the two insects except that with *T. montanus* 2 successive healthy celery and 1 aster plants were used, and with *C. divisa* 1 healthy celery and 1 aster. Table 2 indicates the results obtained.

The percentage of transmission of yellows by *Thamnotettix montanus* to successive healthy celery and aster plants and the recovery of the virus by the same species of leafhopper and by *Cicadula divisa* from experi-

mentally infected celery to healthy celery and asters is given in table 2 and summarized in table 3. With 3 successive sets of celery plants, 9 of the 51 inoculated, or 17.6 per cent, and with 1 set of asters 2 of the 17 plants inoculated, or 11.8 per cent, were infected with yellows by *T. mon-*

TABLE 2

TRANSMISSION OF YELLOWS BY THAMNOTETTIX MONTANUS FROM NATURALLY
INFECTED CELERY TO SUCCESSIVE HEALTHY CELERY AND ASTERS AND
RECOVERY OF VIRUS FROM EXPERIMENTALLY INFECTED PLANTS
BY T. MONTANUS AND CICADULA DIVISA*

Number of T. montanus transferred from infected celery	Successive celery and aster plants inoculated by T. montanus				Virus recovered from experimentally infected celery and transferred				
	Celery			Aster	By T. montanus			By C. divisa	
	First set	Second set	Third set		To celery		To aster	To celery	To aster
					First set	Second set			
10	−	+	+	−	++	++	−−	++	++
10	−	+	−	−	−	−	−	+	+
20	+	−	±	−	+	+	−	+	+
20	+	−	−	+·	−	−	−	+	+
20	+	−	−	+	+	−	−	+	+
20	−	+	−	−	+	−	−	+	+
20	−	+	−	−	−	−	−	+	+
20	−	−	+	−	−	−	−	+	+
20	−	−	−	−	....	....	....	....	....
20	−	−	−	−	....	....	....	....	....
20	−	−	−	−	....	....	....	....	....
20	−	−	−	−	....	....·	....	....	....
20	−	−	−	−	....	....	....	....	....
20	−	−	−	−	....	....	....	....	....
20	−	−	−	−	....	••••	....	....	....
20	−	−	−	−	....	....	....	....	....
20	−	−	−	−	....	....	....	....	...
Total +	3+	4+	2+	2+	5+	3+	0+	9+	9+
Total −	14−	13−	15−	15−	4−	6−	8−	0−	0−
Percentage	*17.6*	*23.5*	*11.8*	*11.8*	*55.5*	*33.3*	*0.0*	*100*	*100*

* The plus sign (+) indicates the production of the disease, and the minus sign (−) shows that no disease resulted. ++ = Virus recovered from 2 sets of experimentally infected celery. −− = Failure to recover virus from 2 sets of plants tested.

tanus that had fed on naturally infected celery plants (table 3). From experimentally infected plants, the virus was recovered by *T. montanus* and transferred to celery from 5 of the 9 infected celery plants, or 44.4 per cent, but was not transferred to any of 9 asters inoculated; it was recovered by *C. divisa* from all of the 9 infected celery plants and transferred to all of the 9 celery and 9 aster plants inoculated, or 100 per cent (table 2).

In another experiment to test transmission by *Thamnotettix montanus* to asters, each lot of 5 or 10 adults of *T. montanus*, after feeding for a period of 3 weeks on celery naturally infected with yellows, was transferred to healthy asters. Four of 15 aster plants inoculated, or 26.6 per cent, were infected with yellows, as shown in table 3. The virus was not recovered from the 4 experimentally infected asters by *T. montanus*, but was recovered by *Cicadula divisa* and transferred to healthy asters and celery.

Bred on Naturally Infected Asters and Celery.—Aster was an unfavorable food plant for the adults of *Thamnotettix montanus*, but the nymphs often acquired the winged stage on large aster plants. Lots of 10, 20, or 25 adults bred on asters naturally infected with yellows were transferred to one or more healthy celery plants and then to healthy asters. Thirty-two celery plants were thus inoculated, and 8, or 25 per cent, became diseased as indicated in table 3. Twenty-two asters were inoculated by the same lots of leafhoppers, but not a single case of aster yellows developed (table 3).

Fifty lots of 20 *Thamnotettix montanus* which had completed the nymphal stages on celery naturally infected with yellows were transferred to healthy celery, one lot to each plant. Fifteen of 50 celery plants thus inoculated, or 30 per cent, developed the disease (table 3).

Fed on Asters and Celery Experimentally Infected by Thamnotettix Montanus.—Nymphs were fed on asters experimentally infected with yellows by *Thamnotettix montanus* and after they acquired the winged stage, each of 25 lots of 5 adults were transferred to a healthy aster, but again all of the plants remained healthy (table 3).

An attempt was made to transfer the virus from asters experimentally infected with yellows by *Thamnotettix montanus* to healthy celery by lots of 50 or 100 males. Each lot of leafhoppers was fed on diseased asters and healthy celery, alternating daily, until all of the insects were dead. With this method the leafhoppers lived from 21 to 42 days. All of the four celery plants inoculated by this method failed to develop the disease (table 3).

Fifteen lots of *Thamnotettix montanus* were fed for a period of 3 weeks or longer on celery experimentally infected by this leafhopper and then each lot was transferred to a healthy aster. One of the 15 asters inoculated, or 6.6 per cent, developed the disease (table 3).

Males of *Thamnotettix montanus* were fed for a period of 3 weeks or longer on 18 celery plants experimentally infected with yellows by *T. montanus* and then each lot of 20 males was transferred to 1 or 2 healthy celery plants. Thirty-four of 70 lots transmitted yellows to 34 of 96 cel-

ery plants inoculated, or 35.4 per cent (table 3). The virus was transferred by *Cicadula divisa* from the original celery plants infected with yellows by *Thamnotettix montanus,* to 14 of the 18 healthy asters inoculated, or 77.7 per cent.

Fed on Asters and Celery Experimentally Infected by Cicadula Divisa.—*Thamnotettix montanus,* after feeding on asters experimentally infected with yellows by *Cicadula divisa,* were transferred to 104 healthy asters, but only 2 asters, or 1.9 per cent, became diseased (table 3). A high mortality of the leafhoppers occurred on small asters, and in all probability the incubation period of the virus in many of the insects was not completed.

Thamnotettix montanus, after feeding on asters infected with yellows by *Cicadula divisa,* were transferred in lots of 20 specimens to 1 or 2 healthy celery plants. Seven of the 39 celery plants inoculated, or 17.9 per cent, became diseased (table 3).

Since a high mortality of the adults occurred on asters, it was decided to feed the leafhoppers for periods varying from 3 to 5 weeks on celery experimentally infected with yellows by *Cicadula divisa.* In one experiment each lot of 5 adults was transferred to 1 or 2 healthy asters and they remained on the plants until all were dead. With twenty-nine lots of 5 insects each, 49 asters were inoculated, but no yellows developed (table 3). In a second experiment lots of 20 leafhoppers were used to inoculate 124 asters, and 3 plants, or 2.4 per cent, developed symptoms of yellows (table 3). Previously noninfective *C. divisa* transferred the virus from the 3 infected asters to healthy asters and celery, but *Thamnotettix montanus* failed to recover the virus. In a third experiment 4 lots of 100 *T. montanus,* after feeding for a period of 27 days on celery experimentally infected with yellows by *C. divisa,* failed to transmit the virus to 4 healthy asters (table 3). In a fourth experiment repeated inoculations of each of 6 healthy aster plants were made by lots of 20 male *Thamnotettix montanus* which had fed for periods varying from 26 to 57 days on celery experimentally infected with yellows by *Cicadula divisa;* when one lot of 20 leafhoppers died on an aster another lot of 20 specimens was put in the cage enclosing the plant, and so on until 5 successive lots of 20 insects were used on each plant. Three lots of 20 males were dead at the end of 1 day on small asters while 1 specimen of another lot lived 18 days. The average longevity of the last living male with 30 lots of 20 leafhoppers was 5 days on small asters. The six asters inoculated by this method remained healthy (table 3).

Lots of 20 *Thamnotettix montanus* were fed for a period of 3 weeks or longer on celery infected by *Cicadula divisa* and then each lot was trans-

ferred to 1 or 2 healthy celery plants. Thirty-six of the 134 celery plants inoculated, or 26.9 per cent, developed symptoms of yellows (table 3).

Fed on Celery Experimentally Infected by Thamnotettix Geminatus. —*Thamnotettix montanus* transmitted yellows from celery experimentally infected by *T. geminatus* to 6 of 26 healthy celery plants inoculated,

TABLE 3

SUMMARY OF RESULTS ON TRANSMISSION OF YELLOWS BY THAMNOTETTIX MONTANUS TO HEALTHY ASTERS AND CELERY

Source of virus	Asters inoculated	Asters infected	Asters healthy	Per cent infected
Collected on celery under natural conditions	12	0	12	0.0
Fed on naturally infected celery	17	2	15	11.8
Fed on naturally infected celery	15	4	11	26.6
Bred on naturally infected asters	22	0	22	0.0
Fed on asters experimentally infected by *T. montanus*	25	0	25	0.0
Fed on celery experimentally infected by *T. montanus*	15	1	14	6.6
Fed on asters experimentally infected by *Cicadula divisa*	104	2	102	1.9
Fed on celery experimentally infected by *C. divisa*	49	0	49	0.0
	124	3	121	2.4
	4	0	4	0.0
	6	0	6	0.0
Fed on celery experimentally infected by *T. geminatus*	19	0	19	0.0
Total	412	12	400	....
Percentage	......	....	......	*2.9*

Source of virus	Celery inoculated	Celery infected	Celery healthy	Per cent infected
Collected on celery under natural conditions	12	2	10	16.7
Fed on naturally infected asters	20	4	16	20.0
Fed on naturally infected celery	51	9	42	17.6
Bred on naturally infected asters	32	8	24	25.0
Bred on naturally infected celery	50	15	35	30.0
Fed on asters experimentally infected by *T. montanus*	4	0	4	0.0
Fed on celery experimentally infected by *T. montanus*	96	34	62	35.4
Fed on asters experimentally infected by *C. divisa*	39	7	32	17.9
Fed on celery experimentally infected by *C. divisa*	134	36	98	26.9
Fed on celery experimentally infected by *T. geminatus*	26	6	20	23.1
Total	464	121	343	....
Percentage	......	......	......	*26.1*

or 23.1 per cent, but failed to transmit the virus to any of 19 healthy asters inoculated (table 3). The virus was not recovered from the 6 celery plants by *T. montanus* but was recovered by previously noninfective *Cicadula divisa* and transferred to healthy aster and celery.

The transmission of yellows from all sources by *T. montanus* to asters average 2.9 per cent and to celery 26.1 per cent as summarized in table 3.

TRANSMISSION EXPERIMENTS WITH SUGAR-BEET CURLY TOP

Since the beet leafhopper, *Eutettix tenellus* (Baker), the vector of sugar-beet curly top in North America, is closely related to the genus, *Thamnotettix*, (it was originally placed in the latter genus [2, 6]) tests were made on whether or not *T. montanus* could transmit sugar-beet curly top. Previously noninfective nymphs or adults, after feeding on curly-top beets, were transferred to 24 healthy beet seedlings, but no curly top developed. Since a high mortality of the leafhoppers occurred on sugar beets, 5 lots of 100 males were fed alternating daily on curly-top beets and healthy celery for a period varying from 1 to 2 weeks, and then each lot of leafhoppers was kept on a healthy beet until the last specimen died. The five beets remained healthy.

ADDITIONAL HOST PLANTS EXPERIMENTALLY INFECTED WITH YELLOWS BY THAMNOTETTIX MONTANUS

Carrot Yellows.—*Thamnotettix montanus* was collected on carrots (*Daucus carota* var. *sativa*) in the Salinas, San Juan, and Sacramento valleys. Previously noninfective leafhoppers, after feeding on 5 carrots naturally infected with yellows, were transferred to 10 healthy celery plants, and 4 of these developed symptoms of yellows. The virus was transferred by previously noninfective *T. montanus* from 2 of these 4 experimentally infected celery plants to healthy celery plants, but attempts to transfer it to asters and carrots were unsuccessful.

An attempt was made to experimentally infect with yellows from celery by means of *Thamnotettix montanus* 3 white, 1 yellow, and 7 orange varieties of carrots. Two plants of each variety were repeatedly inoculated by different lots of leafhoppers. Oxheart or Guerande, an orange variety of carrot, developed typical symptoms of the disease similar to those on carrots infected by *Cicadula divisa* described in a previous paper.[13] The virus was transferred by previously noninfective *C. divisa* from the carrot experimentally infected with yellows to healthy aster and celery plants, but *T. montanus* failed to recover the virus from the carrot.

White London Mustard Yellows.—White London mustard (*Brassica alba*) is a new host plant of aster yellows. This mustard was experimentally infected with yellows by both *Thamnotettix montanus* (in 1 of 2 tests made) and *Cicadula divisa* from the mustard plants experimentally

infected with yellows to healthy asters and celery, but *T. montanus* failed to recover the virus from mustard.

Plants infected with yellows by the two species of leafhoppers developed similar symptoms. The apical leaves were dwarfed, cupped out-
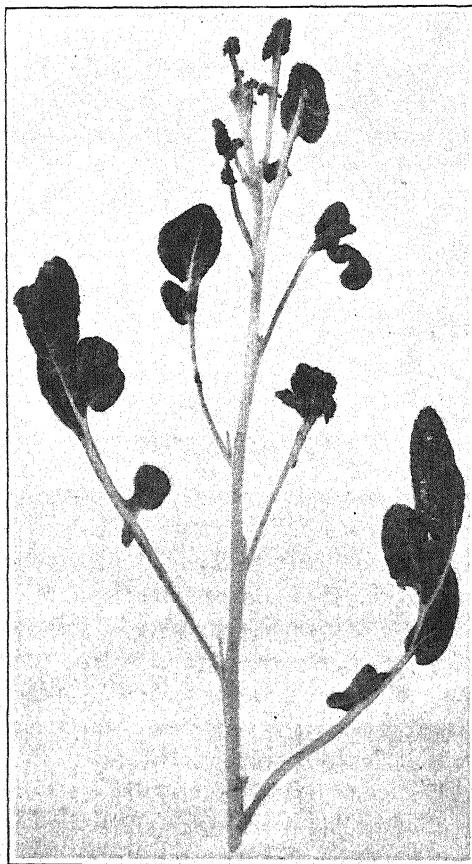
Fig. 1.—White London mustard (*Brassica alba*) experimentally infected with yellows by *Thamnotettix montanus*, showing dwarfed, outward-cupped apical leaves and secondary shoots arising from the axils of the older leaves.

ward, and yellow. Secondary shoots developed from the axils of the leaves (fig. 1).

Prickly Winter Spinach Yellows.—Prickly Winter spinach (*Spinacia oleracea*) was experimentally infected with yellows by *Thamnotettix montanus* (in 1 of 10 tests made) and *Cicadula divisa*. The virus was transferred by previously noninfective *T. montanus* from experimen-

tally infected spinach to healthy celery but not to asters, and by *C. divisa* to both celery and asters.

The symptoms of the disease on spinach infected with yellows by the two species of leafhoppers were similar. The petioles of the outer leaves

Fig. 2.—Prickly Winter spinach (*Spinacia oleracea*) infected with yellows by *Thamnotettix montanus* showing elongated petioles of the outer leaves and many upright secondary shoots with dwarfed leaves and shortened petioles.

were elongated, and many upright secondary shoots developed with dwarfed leaves and shortened petioles (fig. 2).

Prizehead Lettuce Yellows.—Lettuce (*Lactuca sativa*) of the variety Prizehead was experimentally infected with yellows from celery by *Thamnotettix montanus* (only 1 test was made) and developed symptoms of the disease similar to those on lettuce infected by *Cicadula divisa* as described in a previous paper.[13] The virus was transferred by previously noninfective *C. divisa* from experimentally infected lettuce to asters and celery, but *T. montanus* failed to recover the virus from lettuce.

Plantain or Ribgrass Yellows.—In a previous paper[13] plantain or ribgrass (*Plantago major*) was reported to be naturally infected with yellows. Tests were made to determine whether plantain could be experimentally infected with yellows from celery by *Thamnotettix montanus* and whether the leafhopper could recover the virus from infected plantain and transfer it to asters. Previously noninfective *T. montanus* were fed for a period of 36 days on celery experimentally infected with yellows by *Cicadula divisa*. Lots of 20 *T. montanus* were transferred from the celery-yellows plants to 4 healthy plantain plants, and one plant developed typical symptoms of yellows after an incubation period of the disease of 54 days. Seven lots of 5, and 12 lots of 20 *T. montanus* per plant, all failed to transmit the virus from the experimentally infected plantain to 19 healthy asters. On the other hand, previously noninfective *C. divisa* transmitted the virus from experimentally infected plantain to healthy asters..

Tests were also made to determine whether *Thamnotettix montanus* could recover the virus from plantain experimentally infected with yellows by *Cicadula divisa* which had fed on asters or celery naturally infected with the disease. Nymphs of *T. montanus* were fed on diseased plantain until the insects acquired the winged stage and then 10 or 20 males were transferred to healthy asters and celery plants. Often the males were used to inoculate one or more healthy celery plants and then were transferred to one or several asters. Thirty-five asters and 35 celery plants were inoculated from plantain containing the virus originally obtained from asters naturally infected with the disease, but only a single aster developed typical symptoms of yellows. Previously noninfective *T. montanus* recovered the virus from this diseased aster and transferred it to 1 of 4 healthy asters. Twelve asters and 27 celery plants were similarly inoculated from plantain containing the virus originally obtained from celery naturally infected with the disease, but only 1 celery plant developed yellows. The virus was not recovered from the experimentally infected celery plant by *T. montanus,* but was transferred to aster and celery by previously noninfective *C. divisa.*

TRANSMISSION OF YELLOWS BY THAMNOTETTIX GEMINATUS TO HEALTHY ASTERS AND CELERY

Fed on Naturally Infected Asters.—Although *Thamnotettix geminatus* was collected on asters under natural conditions, a high mortality of the adults occurred on small asters in the greenhouse when *T. geminatus* was transferred from large asters in the field. It was found that 6 lots

of 3 leafhoppers fed on 6 healthy asters died within a week, and all of the asters remained healthy as shown in table 6 (p. 356). In all probability the virus incubation period in the insects was not completed.

Bred on Naturally Infected Celery.—Thamnotettix geminatus collected on asters in the San Juan and Salinas valleys deposited eggs in potted celery plants naturally infected with yellows. The nymphs which hatched fed for a period of at least 2 weeks on the celery-yellows plants

TABLE 4

TRANSMISSION OF YELLOWS BY THAMNOTETTIX GEMINATUS TO SUCCESSIVE HEALTHY
CELERY AND ASTERS AND RECOVERY OF VIRUS FROM EXPERIMENTALLY
INFECTED PLANTS BY CICADULA DIVISA*

Number of *T. geminatus* transferred from infected celery	Successive celery and aster plants inoculated by *T. geminatus*						Virus recovered from experimentally infected plants and transferred by *C. divisa*	
	First set of celery	First set of asters	Second set of celery	Third set of celery	Fourth set of celery	Fifth set of celery	To aster	To celery
3	+	−	−	−	+	−	++	++
3	−	−	−	−	+	−	+	....
3	−	−	−	−	+	....	....	+
3	−	−	−	−	−	....	....	....
3	−	−	−	−	+	....	....	+
3	−	−	−	−	+	....	+	....
Total +	1+	0+	0+	0+	5+	0+	4+	4+
Total −	5−	6−	6−	6−	1−	2−	0−	0−

* The plus sign (+) indicates the production of the disease, and the minus sign (−) shows that no disease resulted; ++ = virus recovered from 2 sets of experimentally infected celery.

and were then transferred in lots of 5 or 10 to 6 healthy celery plants. One of 6 celery plants developed symptoms of yellows. After the nymphs acquired the winged stage, 48 adults were transferred to 1 healthy celery plant, which also became diseased (table 6).

Bred on Asters and Celery Experimentally Infected by Cicadula Divisa.—Nymphs lived longer than adults on aster, and sometimes the nymphs acquired the winged stage on asters. Nymphs which hatched from eggs deposited in healthy celery were transferred to asters experimentally infected with yellows by *Cicadula divisa.* Nymphs and adults reared on diseased asters were transferred singly to 35 healthy asters with negative results. Likewise 2 lots of 5 adults and 2 lots of 20 adults failed to transmit yellows to 4 asters (table 6).

Eleven adults of *Thamnotettix geminatus* bred on celery experimentally infected with yellows by *Cicadula divisa* failed to transmit the virus to 11 healthy asters (table 6).

Adult *Thamnotettix geminatus,* collected on asters in the San Juan

and Salinas valleys, were fed for a period of 3 weeks on a celery plant experimentally infected with yellows by *Cicadula divisa*. Six lots of 3 leafhoppers each were transferred to successive healthy celery and aster plants until all of the insects were dead. The adults were fed on each celery plant for a period of 1 week and on each aster for 1 day. The results obtained are indicated in table 4.

Table 4 shows that from the first transfer (to 6 healthy celery plants) infection occurred in 1 plant which developed typical symptoms of yellows; in the second transfer (to asters) all of the plants remained healthy; in the third and fourth transfers (to celery) no infections occurred; and in the fifth transfer (to celery) 5 plants became diseased. In the fifth transfer 4 of the 5 infections occurred at the end of 8 weeks. The minimum virus incubation period in *Thamnotettix geminatus* is not known; in *Cicadula divisa* it was found to be 13 days.[8] The virus was recovered by previously noninfective *C. divisa* and transferred to healthy asters and celery from each celery infected by *T. geminatus*. The virus was also recovered by previously noninfective *T. geminatus* from 1 of the 6 celery plants infected with the yellows by this leafhopper.

Fed on Naturally Infected Celery and Celery Experimentally Infected by Cicadula Divisa and Thamnotettix Geminatus.—Tests were made on the transmission of yellows by lots of 1, 5, 10, 20, and 25 adults of *Thamnotettix geminatus* which were transferred in succession to one or more healthy celery plants. Some of the leafhoppers were collected on various food plants in the field and were fed for a period of 2 to 4 weeks on celery experimentally infected with yellows by *T. geminatus* or *Cicadula divisa*, or on celery naturally infected with the disease. *T. geminatus* which had been bred on celery experimentally or naturally infected with yellows were also used. The leafhoppers were transferred at the end of every 2 weeks to successive healthy celery plants until all of the insects were dead. Table 5 indicates the results obtained on the transmissions of celery yellows obtained with *T. geminatus* but does not show the number of negative tests. Table 5 also shows the recovery of the virus by *C. divisa* from some of the celery plants experimentally infected with yellows by *T. geminatus*, but all of the infected celery plants were not tested.

It is evident from table 5 that *Thamnotettix geminatus* transmitted yellows at irregular intervals, but infections occurred more often on the first celery plant. However, in one case where 15 leafhoppers were transferred to successive healthy celery plants at intervals of 2 weeks until

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[8] Based on unpublished data.

TABLE 5

TRANSMISSION OF CELERY YELLOWS BY THAMNOTETTIX GEMINATUS TO SUCCESSIVE HEALTHY CELERY PLANTS AND RECOVERY OF VIRUS FROM EXPERIMENTALLY INFECTED CELERY BY CICADULA DIVISA*

Number of *T. geminatus* transferred from infected celery	Successive celery plants inoculated by *T. geminatus*								Virus recovered from experimentally infected celery and transferred by *C. divisa*	
	First set	Second set	Third set	Fourth set	Fifth set	Sixth set	Seventh set	Eighth set	To aster	To celery
1	+	....	....	....	....	....	....	....	....	—
1	+	....	....	....	....	....	....	....	....	—
1	+	—	—	....	....	....	....	....	....	—
1	+	—	—	....	....	....	....	....	....	—
1	+	+	—	....	+	....	....	....	....	....
5	—	+	—	+	—	—	+	....	+¶	++§
5	+	—	+	—	....	....	....	....	+	+
5	—	+	+	—	....	....	....	....	+¶	—¶
5	+	—	—	....	....	....	....	....	....	+
5	—	+	—	....	....	....	....	....	+	+
5	+	—	....	....	....	....	....	....	....	+
5	+	+	....	....	....	....	....	....	+	+
5	+	—	....	....	....	....	....	....	....	+
5	+	+	—	....	....	....	....	....	+	+
5	+	—	....	....	....	....	....	....	....	—
5	+	....	....	....	+	....	....	....	+	....
10	—	—	—	—	+	—	—	....	++	+¶
10	+	+	—	—	—	—	—	....	+	+
10	—	+	—	—	—	—	....	....	....	—+
10	+	+	—	—	....	....	....	....	....	+
10	—	—	—	....	....	....	....	....	....	+
10	+	—	—	....	....	....	....	....	....	....
10	+	—	+	—	....	....	....	....	+++	+++
10	—	+	—	+	....	....	....	....	+¶	+
10	+	+	—	....	....	....	....	....	+	++
10	—	—	+	....	....	....	....	....	....	+
10	+	—	—	....	....	....	....	....	....	+
10	+	—	....	....	....	....	....	....	....	....
10	+	+	....	....	....	....	....	....	+¶	....
10	+	+	....	....	....	....	....	....	+	....
10	+	+	....	....	....	....	....	....	+¶	+
10	+	....	....	....	....	....	....	....	+	....
10	+	....	....	....	....	....	....	....	....	—
10	+	....	....	....	....	....	....	....	....	—
10	+	....	....	....	....	....	....	....	....	—
10	+	....	....	....	....	....	....	....	....	....
15	—	—	—	—	—	—	+	+	....	....
15	—	+	....	....	....	....	....	....	+	+
20	+	—	—	—	....	....	....	....	+	....
20	+	—	—	....	....	....	....	....	....	+
20	+	—	—	....	....	....	....	....	+	....
20	—	+	—	....	....	....	....	....	+	+
20	+	—	....	....	....	....	....	....	....	....
20	—	....	....	....	....	....	....	....	+	+
25	+	....	....	....	....	....	....	....	—	....
25	+	....	....	....	....	....	....	....	....	....
25	+	....	....	....	....	....	....	....	....	....
Total +	40+	15+	3+	2+	2+	0+	2+	1+	26+	34+
Total −	13−	25−	28−	13−	5−	7−	3−	0−	2−	15−

* The plus sign (+) indicates the production of the disease, and the minus sign (−) shows that no disease resulted.
¶ Only one test made.
§ Only two tests made.

all of the insects were dead, infections were obtained with the seventh and eighth plants but not with the first 6 plants. This means that an infection was obtained at the end of 14 weeks.

One hundred and ten adults of *Thamnotettix geminatus* were transferred singly to 200 celery plants and only 5 plants, or 2.5 per cent, de-

TABLE 6

SUMMARY OF RESULTS ON TRANSMISSION OF YELLOWS BY THAMNOTETTIX GEMINATUS
TO HEALTHY ASTERS AND CELERY

Source of virus	Asters inoculated	Asters infected	Asters healthy	Per cent infected
Fed on naturally infected asters	6	0	6	0.0
Bred on asters experimentally infected by *Cicadula divisa*	39	0	39	0.0
Bred on celery experimentally infected by *C. divisa*	11	0	11	0.0
Fed on celery experimentally infected by *C. divisa*	6	0	6	0.0
Total	62	0	62	....
Percentage	....	....	....	0.0

Source of virus	Celery inoculated	Celery infected	Celery healthy	Per cent infected
Fed on naturally infected celery	7	2	5	28.6
Fed on celery experimentally infected by *C. divisa*	26	6	20	23.0
Fed on naturally infected celery and celery experimentally infected by *T. geminatus* and *C. divisa*	527	69	458	11.2
Total	560	77	483	....
Percentage	......	....	......	13.7

veloped typical symptoms of yellows. Fifty lots of 5 insects each were transferred to 129 celery plants and 18 positive cases of yellows developed. In the next test 54 lots of 10 leafhoppers each were transferred to 151 celery plants, and yellows was transmitted to 31 plants. A small number of tests were made with larger numbers of leafhoppers as follows: 2 lots of 15 insects each transmitted yellows to 3 of 10 celery plants; 10 lots of 20 insects each to 9 of 32 celery plants; and 3 lots of 25 insects each to 3 of 5 celery plants. A total of 1,205 leafhoppers were tested by transfer to the first set of celery plants. Death of some of the insects occurred in the successive transfers. A total of 527 celery plants were inoculated by means of *T. geminatus* and 69 plants, or 11.2 per cent, developed symptoms of yellows (table 6). The transmission of celery yellows from all sources by *T. geminatus* averaged 13.7 per cent (table 6). The results on the transmission of yellows by *T. geminatus*, as summarized in table 6, show that a total of the 62 asters were inoculated but not a single case of aster yellows developed.

ADDITIONAL HOST PLANTS EXPERIMENTALLY INFECTED WITH YELLOWS BY THAMNOTETTIX GEMINATUS

Carrot Yellows.—Tests were made to determine whether *Thamnotettix geminatus* could recover and transmit the virus more readily from other host plants of yellows. The leafhoppers were commonly taken on carrots in the Salinas and Sacramento valleys. Nymphs and adults after feeding

TABLE 7

TRANSMISSION OF CARROT YELLOWS BY THAMNOTETTIX GEMINATUS AND RECOVERY OF VIRUS FROM INFECTED PLANTS BY CICADULA DIVISA

Variety	Plants inoculated	*T. geminatus* on each plant	Plants infected	Plants healthy	Incubation period in plant, days	Virus recovered and transferred from infected carrots by *C. divisa**	
						To aster	To celery
White varieties:							
Short white	1	5	1	0	33	—	—
	1	15	1	0	30	—	—
	1	20	1	0	36	—	—
	1	25	1	0	37	—	—
	4	1–25	0	4	....	....	....
White Belgian	1	25	1	0	43	—	—
	3	1–25	0	3	....	....	....
Orange varieties:							
Danvers Half Long	5	10–25	0	5	....	....	....
French Forcing	1	10	0	1	....	....	....
Long Orange	9	5–25	0	9	....	....	....
Oxheart or Guerande	7	5–20	0	7	....	....	....
Total	34	....	5	29	....	5—	5—
Average	....	....	....	....	35.8	....	....

* The minus sign (—) shows that no disease resulted.

on carrots experimentally infected with yellows by *Cicadula divisa* or on carrots naturally infected with the disease were transferred to healthy carrots. Table 7 indicates the results obtained.

Table 7 shows that 4 of 8 Short White carrots and 1 of 4 White Belgian carrots were experimentally infected with yellows by *Thamnotettix geminatus*. The leafhoppers failed to infect any of the 4 orange varieties of carrots. The incubation period of the disease in the plant varied from 33 to 43 days, with an average of 35.8 days. The virus was not recovered by *Cicadula divisa* from carrots infected with yellows by *T. geminatus*.

Thamnotettix geminatus failed to transmit yellows to healthy asters

and celery from 5 carrots of an orange variety naturally infected with the disease.

Hollow Crown Parsnip Yellows.—Twelve lots of 3 adult *Thamnotettix geminatus* each, after feeding on Hollow Crown parsnip (*Pastinaca sativa*) infected with yellows by *Cicadula divisa,* failed to transmit the virus to 12 healthy celery plants.

DISCUSSION

If aster and celery yellows are caused by two viruses, then *Cicadula divisa* and *Thamnotettix montanus* failed to separate them, and apparently only one virus is concerned. Host-range differences and overlapping of host ranges have been discussed in a previous paper by the author[15] and by Kunkel[9] and Smith.[17] Among the economic plants infected with California aster yellows by *C. divisa* and by *T. montanus* no host-range differences have been found.

Cicadula divisa transmitted the virus with greater efficiency than *Thamnotettix montanus* or *T. geminatus. C. divisa* transferred the virus from naturally infected asters to 48.3 per cent and *T. montanus* to 20 per cent of the healthy celery plants (table 1). In the recovery of the virus from experimentally infected celery in one experiment, *C. divisa* transferred the virus to 100 per cent of the healthy aster and celery plants while *T. montanus* failed to transmit the virus to healthy asters but transferred the virus to 44.4 per cent of the healthy celery plants (table 2).

SUMMARY

A summary of the results obtained on the transmission of yellows by *Thamnotettix montanus* and *T. geminatus* is given in tables 3 and 6.

It was demonstrated that *Thamnotettix montanus* is a vector of celery yellows under natural conditions.

The transmission of yellows by *Thamnotettix montanus* to asters averaged 2.9 per cent and to celery 26.1 per cent.

Thamnotettix montanus failed to transmit curly top to sugar beets.

The host plants experimentally infected by *Thamnotettix montanus* include, aster, celery, carrots, White London mustard, Prickly Winter spinach, Prizehead lettuce, and plantain or ribgrass (*Plantago major*). White London mustard is a new host plant of California aster yellows.

Thamnotettix geminatus failed to transmit yellows from naturally infected asters, and from asters and celery experimentally infected by *Cicadula divisa,* to healthy asters; but further investigation is being

made on this point. The transmission of yellows from all sources to celery by *T. geminatus* averaged 13.7 per cent.

Thamnotettix geminatus tested singly transmitted yellows to 2.4 per cent of the healthy celery plants.

The host plants experimentally infected with yellows by *Thamnotettix geminatus* were celery and Short White and White Belgian carrots.

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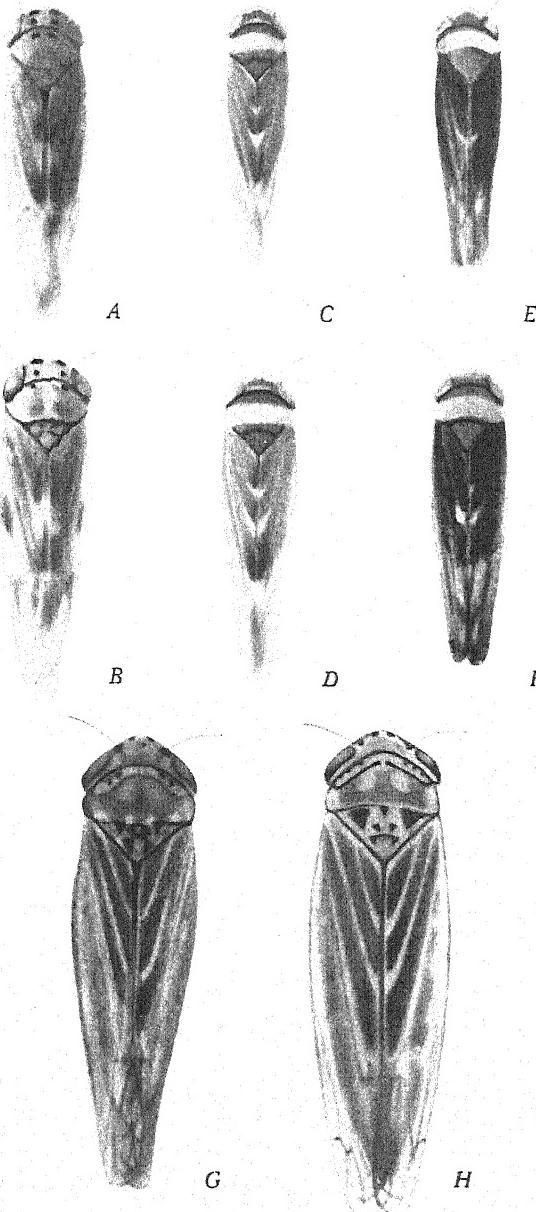
Plate 1.—*A, B, Cicadula divisa; C, D, Thamnotettix montanus,* adults of summer generation; *E, F, T. montanus,* adults of autumn generation; *G, H, T. geminatus.*

BIONOMICS OF
THE WALNUT HUSK FLY, RHAGOLETIS
COMPLETA[1, 2, 3]

A. M. BOYCE[4]

INTRODUCTION

THE PERSIAN WALNUT (*Juglans regia*) industry of the United States is centered in the state of California, where approximately 97 per cent of the tonnage is produced.[4] Batchelor[4] estimates that the bearing acreage for 1930 was 95,900 acres, with an expected increase of approximately 6,000 acres a year for the next three years. Concerning the economics of the industry he states, "Pest control is becoming an increasingly costly operation in some localities, and this is causing the replanting of former walnut acreage to crops not subject to the pests in question."

The recorded insect fauna[3] of the genus *Juglans* numbers over 300 species, only a small number of which occur in California. Before the advent of the walnut husk fly, *Rhagoletis completa* Cresson, the codling moth, *Carpocapsa pomonella* (Linn.), and the walnut aphid, *Chromaphis juglandicola* (Kalt.) were the only species considered to be of major importance to the industry. With the addition of another major pest, the production costs will necessarily be increased in those localities where susceptible varieties are grown.

It is of interest to note that *Rhagoletis completa* is the first species of Trypetidae of major economic importance to become established in California.

The project reported in this paper was undertaken in 1928, when general observations were made on the field biology and economics of the insect. An intensive study of the problem was begun in 1929 and continued through the season of 1932.

HISTORY

This insect has been definitely known to occur in California since 1926,[5] and it may logically be concluded that it was introduced prior to that time. In October of that year S. E. Flanders found dipterous larvae feeding in the exocarp of several varieties of Persian walnut in the Chino section, San Bernardino County. At that time it was suspected that the insect was *Rhagoletis suavis* (Loew), which occurs in eastern United States. Samples of infested nuts were placed under suitable conditions for the insect to complete its life cycle. When adults emerged from the material, they proved to be *Euxesta putricola* Cole, a species with scavenger habits. It was then assumed that the husks of the infested nuts had been mechanically injured and had been subjected to attacks by scavenger species.

However, during the following season (1927) walnuts in the same grove, as well as in several groves that either adjoined or were in close proximity, were noted to have larvae feeding within their green husks. The circumstances surrounding these observations indicated that the insect concerned had deposited its eggs in normal, healthy husk tissue. Again samples were taken for rearing purposes, from which several species of scavenger flies emerged shortly after collection; and the following June (1928) a species of Trypetidae emerged. Specimens of the latter were forwarded to the United States National Museum, where they were identified as *Rhagoletis juglandis* Cresson. This species was described in 1920[11] from specimens reared from larvae feeding in the exocarp of a variety of *Juglans regia* growing on the property of C. R. Biederman in Carr Canyon, Huachuca Mountains, Arizona. The official identification was accepted at that time without further verification.

Since *Rhagoletis juglandis* was reported to be responsible for injury to Persian walnuts in the type locality, a preliminary study of the insect in California seemed advisable. Observations in 1928 showed that the biology of the insect differed materially from that reported by Biederman.[11] He says, "[the fly] appears toward the end of June. . . . The earlier larvae go to the ground by a silk thread, for pupation, but most of them stay in the hull till the nut falls, and pupate in it." Apparently, therefore, much could be gained by visiting Carr Canyon and studying the insect in the locality from which it was first collected and described.

On August 13, 1929, K. L. Wolff and the author reached the homestead of C. R. Biederman in Carr Canyon. Adults were very abundant at that time, and serious damage was resulting from their activity. That the insect in California was entirely different from the one in Arizona was immediately evident. Detailed observations resulted in information contrary to that reported by Biederman. The larvae did not go to the ground by a silken thread, and only a very small number pupated in the walnut husk. Another trypetid was collected at this time from walnuts in Carr Canyon and subsequent study showed that it had not been described. Specimens of flies from Arizona and from California were forwarded to E. T. Cresson for determination. He described the insect from California as *Rhagoletis suavis completa* n. subsp., and the undescribed species from Arizona as *R. boycei* n. sp. These descriptions were published in December, 1929.[12] Cresson examined nine specimens collected in 1917 and 1918 in Texas, that were in the United States National Museum, and considered them to be conspecific with *R. suavis completa*.

One series of specimens in the National Museum, collected at Pecan Bayou, Texas, in 1918, was incorrectly labeled *Rhagoletis juglandis*. Likewise a single specimen from Manhattan, Kansas, in 1921, was incorrectly determined. Through the coöperation of Professor R. C. Smith, the Kansas material was forwarded to the author at the Philadelphia Academy of Natural Sciences, where it was studied in comparison with available type material of the several species of *Rhagoletis* that attack walnut. There were four specimens in the Kansas material collected at Manhattan in 1920: three of them were labeled *juglandis* and the other *suavis*. These four specimens were incorrectly labeled, for they proved to be *completa*. There were four more specimens of *suavis* correctly determined, collected by F. Marlatt, Riley County, Kansas. The date of collection was not given, though circumstances indicate that it was prior to 1910. Large numbers of specimens collected from walnuts in 1930 in Nebraska, Kansas, and Texas, were studied, and *juglandis* was not found in any of this material. Furthermore, all material from Texas was conspecific with that from California, as was the greater portion of that from Kansas, while the remainder from Kansas was *suavis*. The specimens from Nebraska were consistently smaller than *completa* from other sections, though other morphologic differences were not evident. Detailed studies of the systematics of *suavis* and the accepted subspecies *completa* showed that sufficient differences existed in wing markings, male genitalia, and biology to warrant the elevation of *completa* to species rank.

Probable Method of Introduction into California.—In 1930, C. C. Delphey and G. A. Pohl, agricultural inspectors, discovered isolated infestations in wild walnuts at Mountain View, Devore, and Devil's Canyon, San Bernardino County (fig. 1). These infestations are located near the main artery of auto traffic leading into southern California
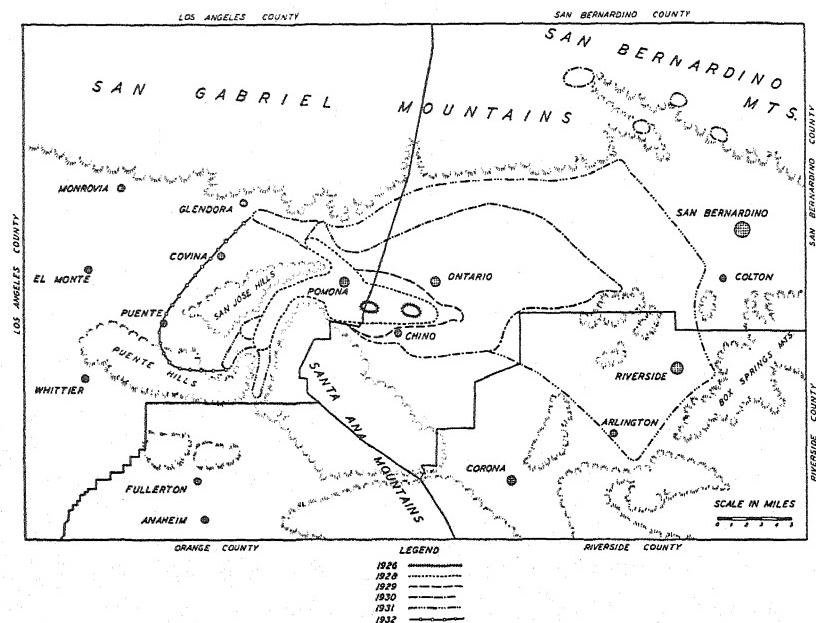

Fig. 1. Distribution of *Rhagoletis completa* in California, showing approximate yearly increase in size of infested area. (Based on survey data obtained from the California State Department of Agriculture and the agricultural commissioners' offices of Los Angeles and San Bernardino counties.)

The rectangle outlined includes only slightly more than that area of each county required to show the extent of infestation. Solid lines within rectangle represent boundary lines of each county.

from eastern states. Therefore it is suspected that auto tourists gathered infested black walnuts when passing through Kansas, Oklahoma, Texas, or New Mexico, and consumed the kernels or otherwise disposed of the walnuts at one of the above locations in California. Since walnuts in the husk have been restricted from entering the state, many lots have been intercepted at the border by the inspecting officers, and in some instances the presence of *Rhagoletis* larvae has been reported. This fact indicates the probable mode of entry.

TAXONOMY AND TECHNICAL DESCRIPTION OF STAGES

Adult.—*Rhagoletis completa* (figs. 2 and 3) was described in 1929 by E. T. Cresson, Jr., who considered it a subspecies of *suavis*.[12] Regarding the insect he states: "This is no doubt a subspecies of the eastern black walnut maggot, in which the median hyaline costal triangle of *suavis* assumes a more or less complete transverse band, extending at least to the fifth vein." His original description follows:

Rhagoletis suavis subsp. *completa* new subspecies.—Of a general ferruginous to tawny color; with a faint median stripe, lateral and posterior portion of the mesonotum, upper portion of pleura including metanotum, forecoxae, all femora beneath and basal portions of second to fifth abdominal segments, brown to black. Frons and occiput medially, mesonotum in general, pectus, abdomen in general, ferruginous. Face, antennae, palpi, posterior orbits, humeri, notopleural stripe, scutellum, apices of second to fourth abdominal segments, femora above, all tibiae and tarsi, yellowish white. Mesonotum medially (leaving lateral margins and posterior area including prescutellar bristles, shining) subopaque, ochreous pollinose, with numerous, short, golden hairs. Bristles of posterior orbits pale. Wings as figured . . . . . . ; similar to *suavis*, but the hyaline area beginning at the costa beyond tip of first vein extends as a transverse band, at least to the fifth vein, generally to the inferior margin. In some specimens there is a diluted spot in the portion of the infuscated band in the apical part of the discal cell, sometimes occupying almost entire apical portion with a streak of same dilution extending through the proximo-median band in discal cell. The median hyaline band often broader than is shown in the figure. Length, 4 to 7 mm.

Type.—Male; Chino, San Bernardino County, California (A. M. Boyce, August 10, 1928; infesting the exocarp of the Persian walnut, *Juglans regia* Linn.), (A.N.S.P. No. 6341). Paratypes. —3 ♀; topotypical (A.N.S.P.). 3 ♂, 4 ♀; topotypical, August 15, 1925 (U.S.N.M.). 2 ♂, 3 ♀; Pomona, Los Angeles County, California, August 10, 1929 (L. Gammon, California State Department of Agriculture).

Subsequently an examination of hundreds of specimens, which were not available to Cresson when he described *Rhagoletis completa,* showed wide variations in certain color characters as well as in the pattern of infuscated areas on the wing of the type specimen. These variations are briefly recorded as follows: Lateral and posterior portion of the mesonotum, upper portion of pleura including metanotum, fore coxae, all femora, pectus, and venter of abdomen may be totally ferruginous or of darker colors ranging to shining black; antennae, palpi, posterior orbits, apices of second to fourth abdominal segments, all tibiae, and tarsi, most commonly ferruginous to tawny in color though frequently the two distal tarsal joints are brownish; halteres, yellowish-white. Hyaline area of wing that begins at costa beyond tip of first vein (R 1) extends as a transverse band at least to third vein (R 4 + 5) though generally to inferior margin. However, in many instances, a narrow, longitudinal, infuscated area at or below third vein (R 4 + 5) connects the two trans-
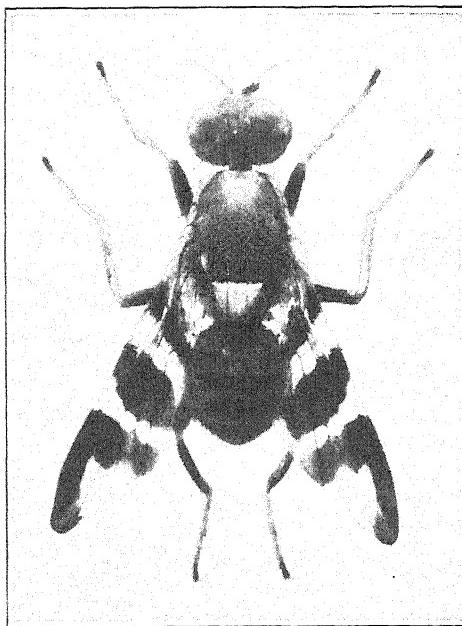
Fig. 2. *Rhagoletis completa*, male, dorsal aspect showing yellowish-white scutellum, wing markings, characteristic position of wings, and rounded tip of abdomen.

Fig. 3. *Rhagoletis completa*, male, lateral aspect, showing yellowish-white lateral stripe on the otherwise dark thorax, extended proboscis, claspers, and anus.

verse infuscated bands, in which case a transverse hyaline band extends beyond to inferior margin. The wing marking is the most important character used in identifying this insect.

The elevation of Cresson's subspecies *completa* to species rank was based on the following differences: Normally *suavis* is appreciably larger than *completa*. The second and third infuscated transverse bands on the wing of *suavis* (fig. 4 *B*) are always joined in the basal portion of the
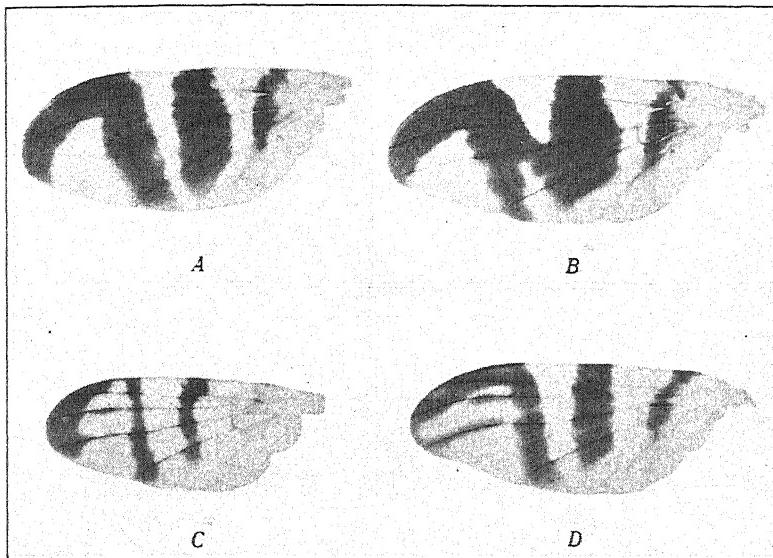

Fig. 4. Left wing of male of the walnut-inhabiting species of *Rhagoletis*, indicating relative size, shape, and pattern of darkened areas. *A, R. completa; B, R. suavis; C, R. juglandis;* and *D, R. boycei.*

first posterior cell (5th R) or in the discal cell (1st M 2). In some instances these two bands are united completely and extend to the inferior margin of the wing as one wide band. However, a relatively narrow infuscated area generally connects these two bands in the discal cell (1st M 2). They separate later and both continue toward the inferior margin. As a result of the union between these two bands a transverse triangular hyaline area is produced, the base of which is a portion of the costal margin, and the apex generally extends at least to the fourth vein (M 1 + 2). It has already been stated that in *completa* the hyaline area transversing the wing between the second and third infuscated bands generally extends to the inferior margin (fig. 4 *A*). Furthermore, the third infuscated band transverses the wing at an angle of approximately 20 degrees or less in *completa*, while in *suavis* the angle has not been ob-

served to be less than 40 degrees, and is commonly less acute. The shape of the wings in these two species differs somewhat, particularly in that a slightly increased degree of tapering toward the apex produces more of a point in *suavis* than in *completa*. The claspers of *completa*, viewed meso-laterally, are somewhat curved and taper gradually from the center toward the distal end, while those of *suavis* are only very slightly curved and taper abruptly at the distal end. The ejaculatory apodeme of the internal male genitalia of these two insects presents important
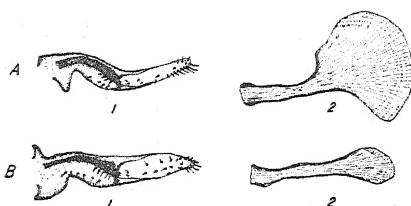
Fig. 5. Comparison of size and shape of claspers (1), and ejaculatory apodeme (2), of *Rhagoletis completa* (*A*), and *R. suavis* (*B*).

differences. In *completa* the distal portion of this structure is broadly rounded and flattened, while in *suavis* it is club-shaped (fig. 5) and only about one-third as wide at its greatest diameter as in the former.
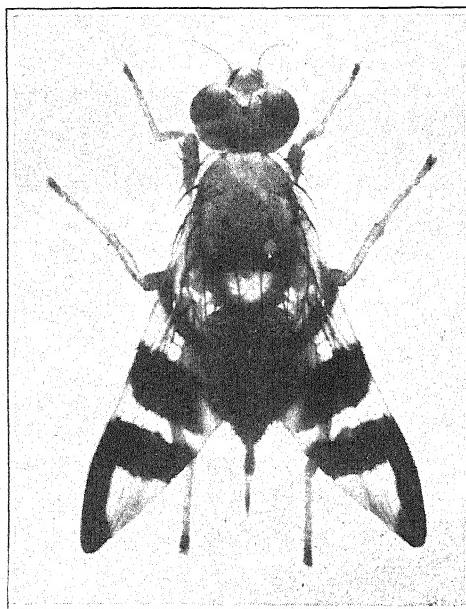
Fig. 6. *Rhagoletis completa,* female, dorsal aspect, showing yellowish-white scutellum, wing markings, characteristic position of wings, and extended ovipositor.

The female (fig. 6) differs from the male only in its slightly larger size, more pointed abdomen, and the presence of the ovipositor.

Egg.—The egg is somewhat curved in shape (fig. 7). It is pearly white when first deposited, becoming darker as the embryo develops. The posterior end tapers slightly and terminates in a very short pedicel, while the anterior end is more pointed. Fine reticulations occur over the entire surface of the shell, though they are more dense on the posterior end. The measurement of 100 eggs supplied the following data: average length 0.96 mm, range in length 0.8 to 1.16 mm; average width 0.22 mm, range in width 0.21 to 0.26 mm.
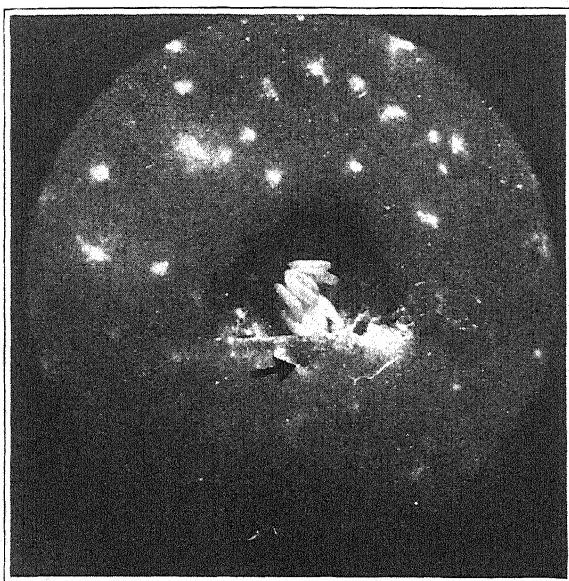
Fig. 7. Photomicrograph of *Rhagoletis completa* eggs in husk tissue of walnut. Arrow indicates puncture made by female in ovipositing.

Larva.—First-instar larvae that are ready to molt range from 1.8 mm to 2.0 mm in length and 0.4 mm to 0.6 mm in width. The body is nearly transparent (fig. 8 *A*), and the large cephalo-pharyngeal skeleton and oral hooks, or mandibular sclerites, are conspicuous because of their dark color (fig. 9 *A*). Each hook bears a prominent tooth-like process located on the blade about midway between the tip and base (fig. 9 *A*). The tracheal system is distinctly visible in detail (fig. 8 *A*). A longitudinal trunk begins at each posterior spiracle and extends forward to the prothoracic segment where it terminates. Anterior spiracles in this instar are lacking. Each posterior spiracle consists of two stigmatic plates, or peritremes through which air enters the trachea (fig. 10 *A*).
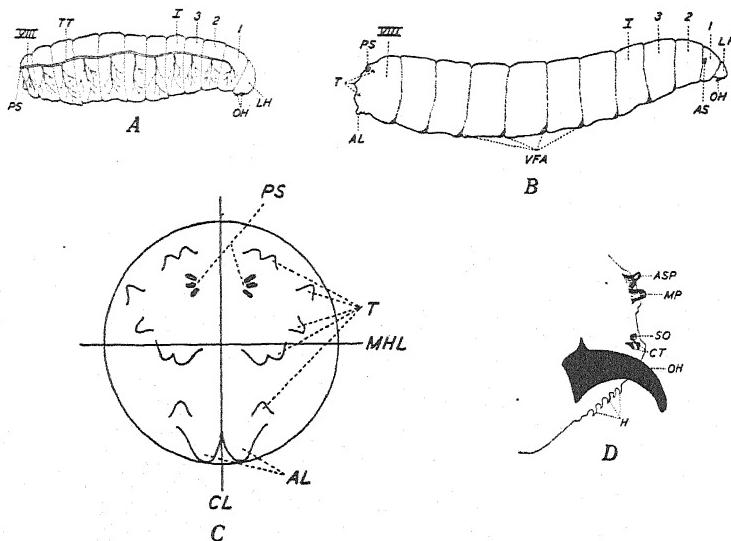
Fig. 8. Structural characters of *Rhagoletis completa* larvae: *A*, first instar, lateral view; *B*, third instar, lateral view; *C*, posterior end, third instar; *D*, head, third instar, lateral view. AL, Anal lobe; AS, anterior spiracle; *ASP*, anterior sense papilla; *CL*, center line; *CT*, chitinized teeth; H, hooklets; LH, larval head; MHL, mesohorizontal line; MP, maxillary palpus; OH, oral hooks; PS, posterior spiracle; SO, sense organ; T, tubercles; TT, longitudinal tracheal trunk; VFA, ventral fusiform areas; 1, prothorax; 2, mesothorax; 3, metathorax; *I*, first abdominal segment; *VIII*, eighth abdominal segment.
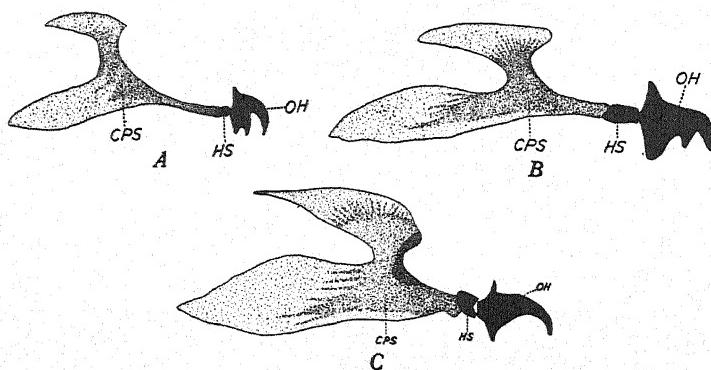
Fig. 9. Mouth parts of *Rhagoletis completa* larvae: *A*, first instar; *B*, second instar; *C*, third instar. CPS, Cephalo-pharyngeal skeleton; HS, hypostomal sclerite; OH, oral hook, or mandibular sclerite.

Four groups of spine-like projections which Efflatoun[13] refers to as "interspiracular processes" are evident on each spiracle.

Second-instar larvae that are about ready to molt range from 4.0 mm to 4.5 mm in length and 1.0 mm to 1.2 mm in width. The body is whitish in color and semiopaque, though dark contents of the alimentary canal are plainly observable. The tooth-like process on each oral hook is retained (fig. 9 *B*). Anterior spiracles occur on the posterior lateral portion of the prothoracic segment, one on each side. They are
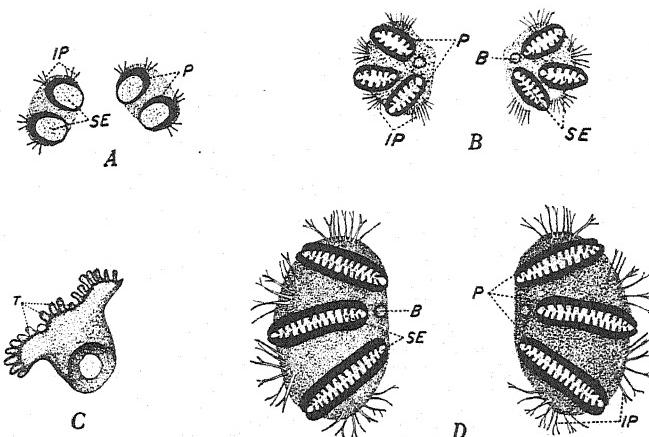

Fig. 10. Spiracles of *Rhagoletis completa* larvae: *A*, first instar, posterior; *B*, second instar, posterior; *C*, second instar, anterior; *D*, third instar, posterior. B, Button; IP, interspiracular processes; P, peritremes; SE, spiracular entrances; T, tubules.

yellowish, somewhat fan-shaped, and possess from 15 to 20 stigmatic papillae, or tubules, arranged in an irregular row on the distal margin (fig. 10). The presence of these spiracles readily distinguishes second-instar larvae from those of the first instar. Each posterior spiracle consists of three peritremes instead of two as previously indicated for first-instar larvae. These structures are yellowish, ovate, and the angle at which each is located with respect to a horizontal plane is characteristic of this species (fig. 10).

Mature third-instar larvae (fig. 8 *B*) average approximately 9.0 mm in length by 2.0 mm in width and they are yellowish-white in color. The tooth-like projection on the blade of each oral hook is absent (fig. 9 *C*). Several prominent structures, other than the oral hooks, occur on the head (fig. 8 *D*). The antennae, or anterior sense papillae, are short two-jointed organs situated on the anterior portion of the head, one on each side. A single-jointed structure, bearing several sensory rods near the

apex, is located just below each antenna. Snodgrass[36] considers these organs as posterior sense papillae, while Efflatoun[13] considers them as maxillary palpi. A pair of small chitinized teeth occur on the side of the mouth, immediately laterad of each oral hook. A sense organ is situated directly above each pair of these teeth, while below them, on each side of the mouth, is a short row of nonchitinized hooklets. The anterior spiracles differ from those found in second-instar larvae in size only. The posterior spiracles are orange-yellow and are slightly raised from the body surface. The peritremes differ somewhat in general shape and relative position to the horizontal plane from those of second-instar larvae (fig. 10 *B* and *D*). The angle at which the lower peritreme is situated with respect to the horizontal appears to characterize this species. The interspiracular processes situated outside and between each peritreme are evident as variable, branching spine-like projections. Ventral fusiform areas are present on nine consecutive segments beginning with the metathorax (fig. 8 *B*). The posterior body segment bears fourteen tubercles, which are characteristically situated and are of considerable importance taxonomically. These structures are shown in figure 8 *C*, following the diagrammatic method used by Greene.[19] Eight tubercles are located above the center line, and the dorsal ones are bifid. Six occur on the lower half of the segment, and those directly below the posterior spiracles are bifid.

Pupa.—The pupa is somewhat barrel-shaped, straw-colored, and measures approximately 5 mm in length by 3 mm in width (fig. 11).
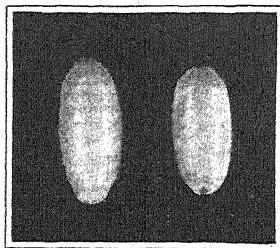
Fig. 11. Pupae, dorsal aspect (left), and ventral aspect (right).

Because of telescoping of the anterior body segments of the larva when the puparium was formed, the anterior spiracles project conspicuously and are dark brown. Both anterior and posterior larval spiracles may be viewed from the dorsal surface. The lateral spiracles of the adult respiratory system are evident on the mesonotum and all abdominal segments. The circular cleavage line is located approximately in the middle of the first abdominal segment and encircles the entire puparium, though it is most prominent on the dorsal half. The horizontal cleavage line connects with the circular cleavage line at right angles on each side and extends around the anterior portion of the puparium in a midlateral plane. On the venter the larval mouth and anal opening appear as dark-brown, invaginated areas. The ventral fusiform areas are present, though not conspicuous.

RELATED SPECIES ATTACKING WALNUTS

Four members of the genus *Rhagoletis* have as primary hosts species of *Juglans.* All are probably indigenous to the continent of North America. The insects are: *R. completa, R. suavis, R. juglandis,* and *R. boycei.* A brief discussion of the last three species follows:

Rhagoletis Suavis.—*Rhagoletis suavis* (Loew) (fig. 12) is the largest species of the genus and was described in 1862 by Loew[24] under the old genus *Trypeta.* It is commonly known as the walnut husk maggot.

Fig. 12. *Rhagoletis suavis,* male, showing characteristic wing markings and yellowish-white lateral stripe on otherwise yellow thorax.

Prior to 1930 this species was recorded from Massachusetts, Connecticut, New York, Pennsylvania, Maryland, West Virginia, North Carolina, South Carolina, Ohio, Indiana, Illinois, and Minnesota. Since then new records have been obtained, in which instances preserved specimens of larvae or adults, or both, have been determined by the author. These records, together with the authority, are as follows: Manhattan, Kansas, 1930 (R. Smith); Ames, Iowa, 1930 (Beck); Sturgis, Mississippi, 1930 (Myers); Bloomfield Hills, Michigan, 1931 (Ries); Fayetteville, Arkansas, 1931 (Baerg); and Columbia, Missouri, 1932 (Haseman). The present known distribution is shown in figure 13. The distribution of this insect probably conforms to the range of the eastern black walnut, *Juglans nigra,* and the butternut, *J. cinerea.* Brooks[8] has also

reared this insect from the Persian walnut, *J. regia,* and the Japanese walnut, *J. sieboldiana.* It is reported to be of economic importance in New York,[17] Pennsylvania, and Maryland in relatively small plantings of Persian walnuts. This species would probably constitute an economic problem should it become established in the commercial walnut-producing areas of the West.
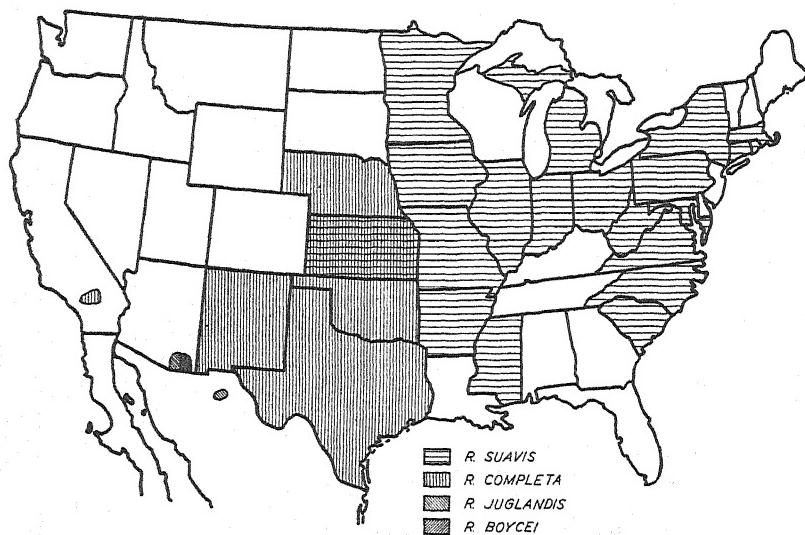
Fig. 13. Distribution of the walnut-inhabiting *Rhagoletis.*

Rhagoletis Juglandis.—Rhagoletis juglandis Cresson (fig. 14) was described in 1920 by Cresson[11] from material collected by C. R. Biederman, the larvae of which were feeding in the green husks of Persian walnuts, and Arizona black walnuts, *Juglans rupestris,* in Carr Canyon, Huachuca Mountains, Arizona. Van Duzee collected this fly from Badger, Arizona. There are no other records from the United States, and those reported are close together and within a few miles of the Mexican border. Beck collected this insect on black walnut at Colonia Dublan, Chihuahua, Mexico, in 1931 (fig. 13). It is probably of Mexican origin. The adult is considerably smaller than *suavis* or *completa* and almost entirely yellowish in color. Observations on its field behavior, made in this study, indicate that this species is the most active one of the group attacking walnuts. When visiting Carr Canyon in July, 1930, J. C. Caldwell found that the adults had apparently emerged earlier than usual. He obtained second-instar larvae that were feeding in the developing walnut kernel since the shell had not hardened sufficiently to

prevent penetration. It appears that this species is probably capable of causing serious losses if established in commercial walnut-producing areas.
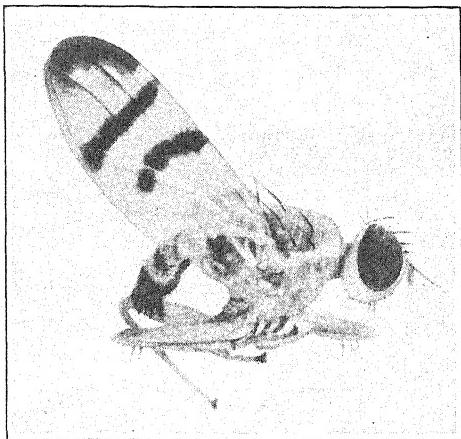
Fig. 14. *Rhagoletis juglandis*, male (topotype), showing relatively small size, characteristic wing markings, and yellowish-white lateral stripe on otherwise yellow thorax.
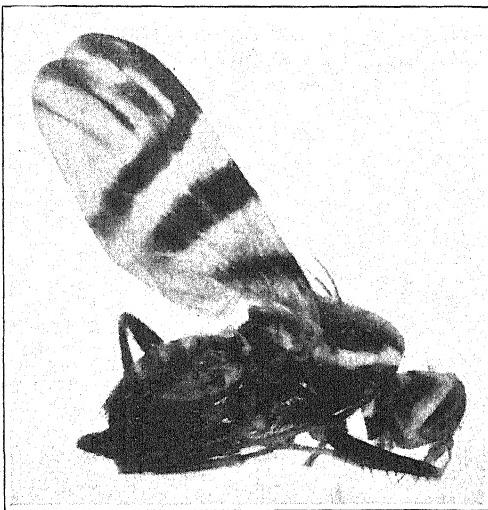
Fig. 15. *Rhagoletis boycei*, male (topotype), showing relatively large size, characteristic wing markings, and yellowish-white lateral stripe on otherwise black thorax.

Rhagoletis Boycei.—Rhagoletis boycei Cresson (fig. 15) was described by Cresson[12] in 1929 from material collected by K. L. Wolff and the author from Carr Canyon, Huachuca Mountains, Arizona (fig. 13).

Here it occurred with *juglandis*, though only a few specimens were observed at that time. In one instance a female was collected while ovipositing in the green husk of a Persian walnut, which fact indicates that this walnut is probably a host. Little is known regarding the distribution and biology of this insect. Limited observations in the type locality indicate that it is very wary and wild, and possibly has habits approaching solitude. Therefore an opinion regarding its possible economic importance is withheld.

COMMON NAME

The matter of a satisfactory common name for *Rhagoletis completa* has received much consideration. Early in this study, when the species had been determined erroneously as *R. juglandis*, it was evident that the common name "black walnut fly" by which it had already been referred to in literature[11] was not suitable. A committee was appointed by the Entomological Club of Southern California to consider common names and was instructed to submit the most satisfactory name to the American Association of Economic Entomologists for adoption. In view of the generally accepted concepts of a satisfactory common name, and because a "walnut husk maggot" already existed, the name "walnut husk fly" was proposed and was officially accepted.[1]

In the light of present knowledge, this official common name was actually intended for *Rhagoletis completa*,[5] instead of *R. juglandis*. The author accepts this version of the matter, and the insect is known as the walnut husk fly among entomologists, walnut producers, and others who have occasion to use a common name in California.

DISTRIBUTION

Rhagoletis completa is apparently indigenous to mid and south central United States (fig. 13) and its known distribution does not extend very far east of the one hundredth meridian. Since the correct identity has been established,[12] knowledge regarding distribution has been greatly increased. At the time of Cresson's study of this species from California in 1929, it was known, though erroneously determined, from but one other state—Texas. It was collected at Brownwood, Texas, by A. I. Fabis, in 1917, and from Pecan Bayou, Texas, in 1918 by the same collector. The coöperation of an entomologist in each state of the Union was solicited, and as a result new records were secured for this species as well as for *suavis*. In all instances the coöperators have kindly forwarded preserved specimens of larvae or adults, or both, to the author for determination. The records obtained for *completa*, and the authority, are as follows: Lincoln, Nebraska, 1930 (Swenk); Manhat-

tan, Kansas, 1930 (R. Smith); Stillwater, Oklahoma, 1931 (Sanborn); Comanche, Texas, 1930 (Nickels); and State College, New Mexico, 1932 (Eyer). Entomologists report personal knowledge of the existence of this species in Kansas, Oklahoma, and Texas for thirty to fifty years. *R. completa* has not been collected east of the ninety-fifth meridian, and there is but one record of *suavis* west of the ninety-seventh. At Manhattan, Kansas, both species were taken from the same tree. The ratio of *completa* to *suavis* adults obtained from extensive rearings there in 1932 was approximately 50:1. This probably indicates the operation of factors which limit the western distribution of *suavis*, while conditions for *completa* apparently approach optimum. In all records outside California the host of *completa* has been listed as wild (black) walnut; however several species are probably involved.

In California the insect is recorded from Los Angeles, San Bernardino, and Riverside counties. A relatively small area in each county is infested, the total constituting approximately 500 square miles. The total infested acreage of commercial walnut groves at the end of the 1932 season was approximately 2,000 acres. The locations of the first recorded infestation in 1926, together with other data regarding distribution, are shown in figure 1.

WALNUT AS A HOST

"Host" as used in this study means any plant that serves to support the insect under any condition through the egg and larval stages, provided larvae so developed produce normal pupae and adults.

This species is practically monophagous, confining its attack almost entirely to the genus *Juglans*. During the course of these investigations infestations have been recorded from every species of *Juglans* and from practically all varieties of Persian walnut, *J. regia*, found growing within the infested area. The wild species and hybrids are: southern California black walnut, *J. californica*; northern California black walnut, *J. hindsii*; eastern black walnut, *J. nigra*; paradox hybrid walnut, *J. regia × J. hindsii*, and Royal hybrid walnut, *J. hindsii × J. nigra*. The cultivated varieties of Persian walnut are listed in two groups according to the degree of susceptibility to attack.

Very susceptible	Slightly susceptible (resistant)
Eureka	
Franquette	Placentia
Mayette	Seedling (most types)
Klondike	Ehrhardt
Payne	Ware
Seedling (certain types)	Neff

Husk Hardness as a Factor in Varietal Susceptibility.—Early in the history of the walnut husk fly in California, a very pronounced difference in varietal susceptibility to attack was observed. An instance was recorded in 1928 in which over 95 per cent of the walnuts on Eureka trees were infested, while those on Placentia trees alternately planted among the Eureka trees were only 2 per cent infested. Observations indicated that the later-ripening, thick-husked varieties were most favored as hosts. Since the flies oviposited in the green husk tissue, varietal susceptibility appeared to be related to the hardness of the husk at the time of oviposition activity. In order to obtain information regarding this matter, studies were conducted to determine the pressure in grams required to puncture the husks of the several varieties of walnuts during the oviposition period.
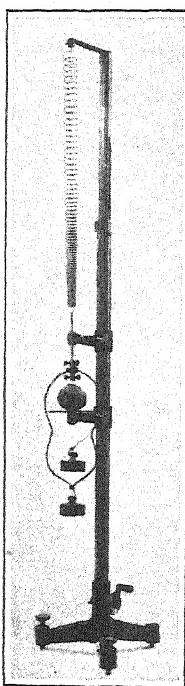
Fig. 16. Jolly balance modified for use in determining the pressure required to puncture green husks of walnuts and the skins of various fruits.

Method of Testing Husk Hardness.—A modified Jolly balance (fig. 16) was used to determine husk hardness. This instrument consists essentially of a spring with one end attached to the arm of an upright extensible column while the other end is attached to a steel puncturing rod which also has weights indirectly connected by means of a stirrup-shaped frame. The puncturing rod assembly has a horizontal hair line on that portion which connects to the lower end of the spring. This portion of the rod passes through a short piece of glass tubing that is mounted on the frame and which also has a hair line at its center. The puncturing rod used was cylindrical with a tip that was flat in cross section. A stage on the upright column supports the material that is to be tested for hardness. The extensible column is graduated and a vernier scale is mounted on the stationary column to permit accurate reading.

The technique of operation is as follows: Tension is applied on the spring by extending the inner portion of the upright column by means of a rack and pinion adjustment. When the tension is sufficient to balance the attached 400-gram weight plus the weight of puncturing rod and rigging, adjustment is continued until the hair line on the metal rod coincides with that on the glass tube through which it is suspended. The walnut to be tested is placed on the stage. Then the stage is adjusted so that the tip of the puncturing rod is barely touching the spot

on the husk to be punctured. With the walnut held firmly in place the tension on the spring is steadily reduced until the rod penetrates the husk. Penetration is indicated by a sudden drop of the flat-tipped rod into the tissue.

The reading on the graduated column at the point where penetration occurred is taken and subtracted from the zero (reading when spring tension perfectly balances weight, and hair lines on metal rod and glass tube coincide), which permits the calculation of the weight in grams required to puncture the green husk. When the cross-sectional area of the puncturing rod is known, the actual pressure in grams required per square millimeter to puncture the husk may be computed. The rods used in 1929 and 1930 became inadvertently mixed with others before their respective areas were computed. Therefore the data given for husk hardness during those two years are only relatively comparable to other data presented. However, the same rod was used throughout a single season, and thus all the data for any one season are strictly comparable. In the 1931 and 1932 studies the same rod was used throughout and the area computed, thereby permitting comparison of husk hardness on the basis of grams pressure required to puncture one square millimeter of husk surface. The area of the puncturing rod was 0.2243 sq. mm.

In the tests of 1929 and 1930, samples of 25 walnuts were selected at random from each variety. Two puncture readings were made of each walnut, one for the stem region and one for the middle region. Thus 50 readings were made per test for each variety. Preliminary puncture data showed that the calyx region was materially harder than other regions; therefore this region was disregarded in these tests. In the tests of 1931 and 1932 each random sample of a variety consisted of 50 walnuts, and 12 punctures were made per walnut, 4 punctures placed in each of the stem, middle, and calyx regions. The punctures in each region were equally spaced on the circumference. Thus the number of readings per test per variety was 600.

Results of Husk-Hardness Tests in 1929.—The data obtained in 1929 are graphically presented in figures 17 and 18.

Figure 17 shows that the husks of Placentia walnuts are considerably harder than those of the Eureka variety. The former is one of the least susceptible, while the latter is one of the most susceptible varieties. The reason for the softened condition of the husk during the middle of August is not known. Husks soften as the walnuts approach the ripening condition. Most of the eggs were deposited during late August and the fore part of September (fig. 59). Normal harvest for the Eureka variety begins about October 15, while the harvest for the Placentia variety begins about September 15. Therefore the period intervening
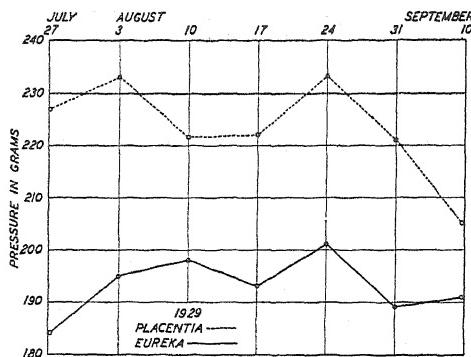
Fig. 17. Pressure in grams required to puncture the green husk of Placentia and Eureka walnuts during the period of activity of *Rhagoletis completa* (1929).
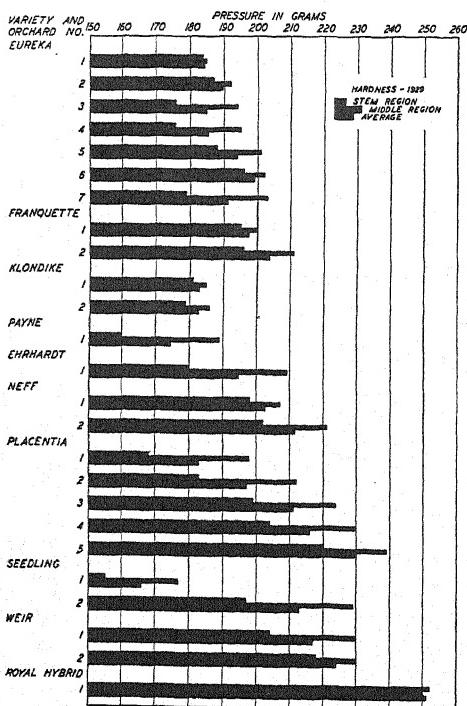
Fig. 18. Husk hardness of various walnut varieties, together with variation in hardness of the same variety in different groves. Data collected August 25–28, 1929.

between the time that Placentia husks reach a susceptible state and the time of harvest is too short for appreciable larval development.

The data presented in figure 18 show a wide range of husk hardness with different groves of the same variety. The differences are probably due to differences in orchard management together with local conditions influencing the developing crop. From figures 18 and 59 it is evident that oviposition in Eureka walnuts took place when a pressure of approximately 190 grams or less was required to puncture the husk. On this basis the walnuts in several orchards of susceptible varieties were not in a susceptible condition at this time and subsequent observations showed them lightly infested. Previous observations had shown that occasional orchards of susceptible varieties were lightly infested despite the presence of many flies. Furthermore infestation of the Franquette variety usually takes place later than that of other varieties. The data indicate that certain resistant varieties, such as Ehrhardt, Placentia, and Seedling, were in susceptible condition for infestation. In these instances the early harvest would have prevented any appreciable amount of larval development.

It is particularly interesting to note that in every instance with the Persian walnut the stem region was softer than the middle region. Oviposition data in 1928 showed that 70 per cent of the egg cavities were located in the stem region.

Irrigation and Susceptibility to Infestation.—Field observations and limited field data indicate that, other factors being comparable, irrigation practices have a bearing on susceptibility to infestation. An abundant supply of water throughout the growing season apparently increases susceptibility. In 1927 and 1928 the trees nearest irrigation outlets and those at the low end of the run of water where flooding takes place, were the first infested and were usually more heavily infested than those receiving less water. Furthermore several instances are recorded where nuts on trees of the Placentia variety (normally resistant) were apparently rendered susceptible to the extent that 25 per cent or more were infested as a result of being located within a few feet of a leaky irrigation stand. The inference was that perhaps abnormal amounts of water resulted in more succulency in the tree, rendering the husk softer and thereby creating a condition more favorable to oviposition. In one instance data obtained from trees in a certain grove of the Eureka variety where the soil received excessive amounts of water at each irrigation showed that an average pressure of 187.6 grams was necessary to puncture the husks while 203.1 grams was necessary where trees received normal amounts of water. Further studies regarding irrigation and susceptibility to attack were conducted in 1931.

Husk-Thickness Studies in 1929.—Limited studies were made in 1929 on husk thickness of the more common varieties. A steel millimeter scale with beveled tip was employed for this purpose. The tip of the scale was inserted into the green husk, at a right angle to the surface, until the shell of the nut was reached, at which point the thickness of the husk was read in tenths of a millimeter. The measurements were made on the same walnuts that were used in the husk-hardness tests. Two measurements were taken in each stem and middle region. Preliminary measurements showed that the husk was consistenly thinnest in the calyx region. Data were not collected for this region since eggs are rarely deposited in this location. A mean of 100 readings per variety constituted the data presented in figure 19.
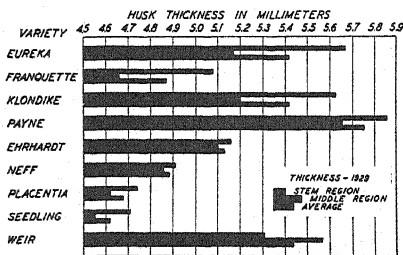
Fig. 19. Husk thickness of the common walnut varieties in the area infested by *Rhagoletis completa*. Data collected August 25–28, 1929.

The data do not conclusively show that a relation exists between husk thickness and susceptibility to infestation. However, it is interesting to note that the most resistant varieties possess a thinner husk than the very susceptible ones, and furthermore that the ratio of thickness of stem region to middle region in the very susceptible varieties is generally greater than in the resistant ones. The fact that approximately 70 per cent of the egg cavities observed were located in the stem region indicates the possibility of a relation between thickness and location of egg cavity which would suggest the general relation between thickness and hardness.

Results of Husk-Hardness Tests in 1930.—Data were obtained in 1930 regarding the husk hardness of the most important commercial varieties in both infested and uninfested walnut-producing areas of the state. These data are presented in figure 20. In all instances the data show that the stem region is softer than the middle region.

Conclusions regarding the probable degree of susceptibility of varieties growing in different sections of the state are unwarranted. The time

of ripening of any given variety changes with the locality. Therefore important differences in husk hardness at the time of oviposition are to be expected. Furthermore, the effect of climatic conditions in various sections of the state upon the time of emergence of the fly is unknown. Should flies emerge in early June it is probable that the walnut husks would not have attained sufficient hardness to prevent oviposition. More-
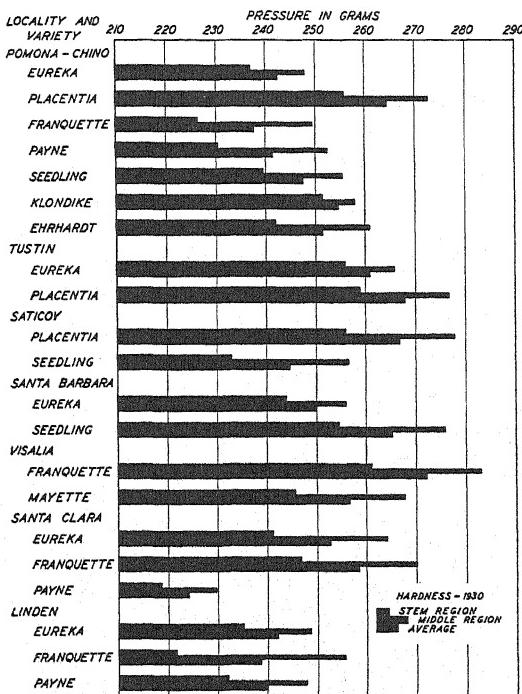
Fig. 20. Husk hardness of the more important varieties of Persian walnuts from the walnut-producing areas of California. Data collected August 20–25, 1930.

over if the time of adult emergence was not materially altered it is entirely probable that the most susceptible varieties growing in those sections where maturity is reached relatively later in the season may be attacked before the husk hardens sufficiently to prevent oviposition. Should the walnut become infested before the shell is formed and hardened, the larvae would no doubt consume the kernel in feeding.

Results of Husk-Hardness Tests in 1931.—The husk-hardness studies were continued in 1931 and were considerably enlarged upon with respect to the effect of irrigation practices upon husk hardness and subsequent degree of susceptibility of Eureka walnuts to infestation. These

husk-hardness irrigation data more particularly concern economic control and are for that reason treated elsewhere under 1931 field control plots, experiment XVII, page 549. The data of 1931, comparing husk hardness of various varieties, are presented in figure 21.
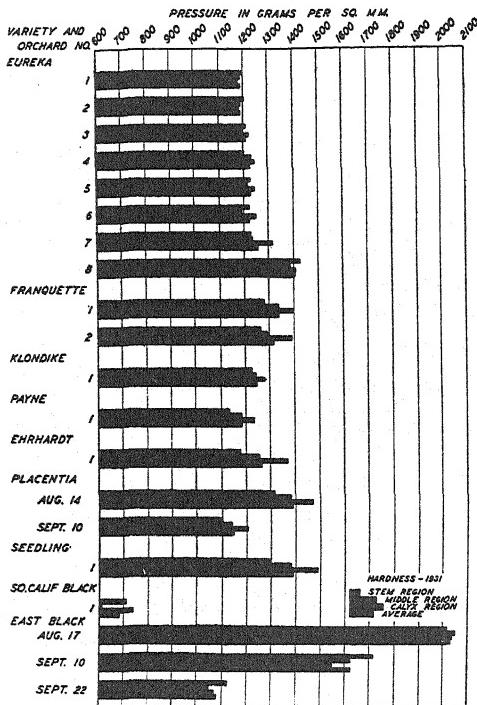
Fig. 21. Husk hardness of various varieties of walnuts, showing variation in hardness in various groves and on different dates. Data collected August 18–22, 1931, unless otherwise indicated.

The Eureka walnuts from various orchards were remarkably uniform in husk hardness with the exception of Orchard No. 8. This orchard adjoins Orchard No. 1 on the south. It is of especial interest to note that irrigation is not practiced in Orchard No. 8, while it is an important factor in the orchard practices of most commercially produced walnuts. The degree of infestation in Orchard No. 8 was very light, despite the fact that large numbers of flies were liberated in the grove experimentally. Observations in this grove over a period of five years have shown that a consistently light infestation exists even when the infestation in the adjoining grove has on several occasions reached 90 per cent.

The data given for the Placentia variety are from the same grove, though obtained on different dates. They show that the husk becomes appreciably softer as the nuts approach ripening. A similar relation is shown by the data for the eastern black walnut.

In most instances the 1931 data for the Eureka variety indicate very slight differences in hardness between the three regions of the husk. Furthermore, in most instances the middle or calyx region is softer than the stem region, which is contrary to the 1929 and 1930 data. With the other Persian varieties the ascending order of hardness is stem, middle, and calyx regions, which is in accord with data obtained in previous years.

Results of Husk-Hardness Tests in 1932.—The observed differences in hardness of the various regions of Eureka walnut husks led to further studies in 1932. A comparison was made between the hardness of the three husk regions of the Placentia and Eureka varieties extending from July 16 to September 7. The data obtained, indicating the trend of husk hardness throughout the seasonal activity of the fly, are presented in figure 22.

The data for 1932 show that the stem region in Eureka walnuts is appreciably harder than either the middle or calyx regions, while the reverse condition exists with the Placentia variety. The average hardness of the latter variety is materially greater than that of the Eureka variety. This fact substantiates the earlier work of 1929 and is probably the most important factor governing the wide difference in susceptibility to attack of these two varieties.

Importance of Husk Hardness as a Factor in Susceptibility.—The most important factor pertaining to the susceptibility of varieties of Persian walnuts to attack by the walnut husk fly is that of hardness of the husk. Husks of Persian walnuts increase in hardness as the nuts increase in size and age, reaching the peak of hardness usually in late June, after which they become softer as

Fig. 22. Husk hardness of Placentia and Eureka walnuts in 1932.

maturity is approached. The degree of hardness when the peak is reached and the extent of subsequent softening appears to be a varietal

characteristic. As a result of the physical nature of the husk of the resistant varieties, the fly is unable to penetrate the surface in order to deposit eggs. Under both laboratory and field conditions females have frequently been observed to fail in their attempts to puncture the husks of the less susceptible varieties. Furthermore they are unable to puncture the husks of susceptible varieties until a certain degree of maturity and resultant softness has been reached.

Owing to individual variation in nuts upon a single tree with respect to husk hardness, some nuts are in a susceptible condition throughout the entire period of fly activity. The female fly apparently finds the susceptible walnuts and also the location for the egg cavity by "trial and error." Females are frequently observed attempting oviposition at many places on walnuts without success, before finally succeeding in penetrating the husk of a walnut and depositing a batch of eggs.

PEACH AS A HOST

From laboratory rearing records and certain field studies the peach, *Amygdalus persica,* has proved to be a host. Eggs were deposited and larvae reached maturity in the Elberta, Simms, Lovell, and Phillips varieties in a battery-jar cage. Also the immature stages were completed when adults were confined on a tree of the Lovell variety by means of a $12 \times 12 \times 12$ foot cheesecloth cage over the entire tree. The varieties mentioned mature relatively late in the season during the height of fly activity.

Flies were commonly observed on peach trees in the vicinity of infested walnut trees. Natural infestation has been recorded in several instances where peaches were growing as interplants with Eureka walnut trees that were infested. However, the average number of larvae maturing in individual peaches under both field and laboratory conditions was relatively few, which indicates that this host does not afford optimum conditions for development. Adults emerging from larvae that developed in peach were apparently normal in all respects. It appears that at least some peaches from trees growing interplanted with infested walnuts are harvested with eggs and young larvae in them. The period elapsing between the time when peaches are in a physical condition favorable to oviposition and the time of normal harvest is too short for an appreciable amount of larval development to take place. The observed infestations under natural conditions were in tree-ripe peaches that had not been harvested. Hardness tests on unripe peaches from one interplanted grove showed that 245 grams pressure was required for penetration, while 193 grams was required for the Eureka walnut inter-

plants. At that time the peaches were not infested but the walnuts were. Later, as the peaches approached ripeness and became softer, they were infested, but no data on hardness were obtained.

STUDIES ON POSSIBLE HOSTS

Laboratory studies were conducted with the object of determining whether or not certain fruits would serve as hosts for *Rhagoletis completa*. In a few instances tests were conducted in the field. The general procedure of the laboratory tests was to confine from 25 to 50 gravid females and the same number of males in an inverted battery-jar cage containing the fruit to be tested. Usually 12 tests were made with each fruit. Sucrose was included in each cage as food. Each test extended over a period of several weeks. The behavior of the flies in the various tests was observed several times daily. The host material was removed periodically and inspected for the presence of eggs. When oviposition had
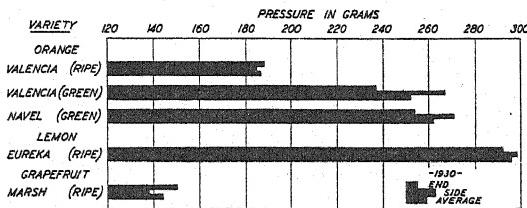
Fig. 23. Rind hardness of certain citrus fruits. Data collected September 17, 1930.

occurred the fruit was placed in a battery jar and kept under conditions favorable for the development of the insect. Sifted sand was placed in the bottom of each battery jar so that any larva maturing could pupate. The sand was again carefully sifted at the termination of the test to note whether or not pupae were present.

Several fruits and vegetables commonly grown in the infested district were tested.

Orange.—The navel orange (*Citrus sinensis*) is eliminated as a possible host because it ripens in the winter season and the green oranges are too hard for the females to puncture, as shown by data on skin hardness (fig. 23) and by laboratory studies of the behavior of adults when confined with green navel oranges.

In numerous laboratory experiments conducted with ripe Valencia oranges, sexually mature flies were confined for weeks with the oranges. In every instance females oviposited, though at no time were the eggs inserted deep enough in the skin to hatch before they dried up. They

punctured the rind of the orange rather readily (fig. 24) and went through the actions of normal oviposition, except for the fact that they were unable to lacerate the inner tissue of the rind to make a cavity in which to place the eggs. Instances were observed where females worked vigorously for half an hour trying to make an egg cavity, without success. Often a single egg was deposited in a puncture and others were placed on the surface near the hole.

In several of the tests Eureka walnuts were placed in the cage with the oranges. The flies remained on the oranges almost exclusively,
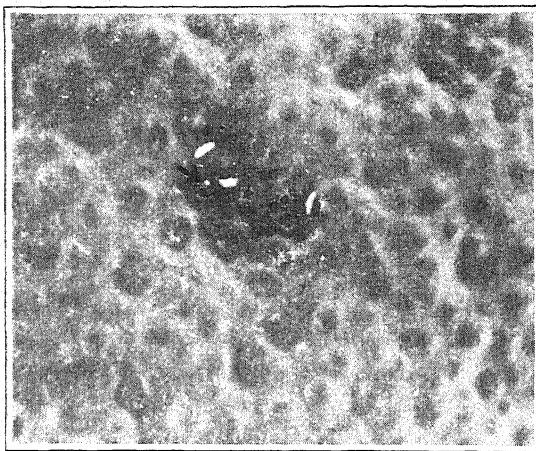
Fig. 24. The surface of a Valencia orange, with punctures representing many attempts to oviposit; and four eggs, three deposited on surface and one, indicated by arrow, nearly submerged in rind tissue.

actively attempting oviposition and directing no attention to the walnuts until the oranges were removed from the cages.

In a field test a cheesecloth cage (12 × 12 × 12 feet) was built over a small Valencia orange tree bearing a good crop of ripe and also green fruit. A total of 500 flies, some newly emerged and some sexually mature, was liberated on this tree at varying intervals of time. To insure an adequate supply of food, sucrose-sweetened water was sprayed on the foliage. The flies appeared to behave normally. At the end of the season all oranges were sliced and examined for the presence of larvae. None was found; however, a few oranges exhibited evidence of attempts at oviposition by the females.

In laboratory tests involving a total of 50 oranges, eggs were artificially placed in the tissue below the skin. In 4 of these hatching took place and the larvae reached maturity. In 1 orange several larvae pu-

pated within the tissue, while the others emerged through the hole where the eggs were injected. When early-stage larvae were placed within orange tissue they apparently developed in a normal manner and reached maturity. The foregoing data indicate that the Valencia orange might serve as a host if females were capable of depositing their eggs normally. However, it should be pointed out that the identity of pupae of larvae
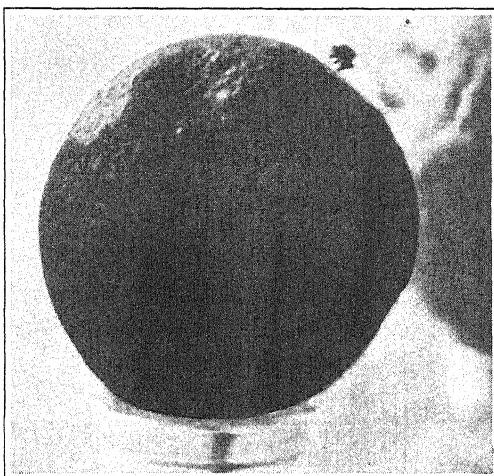
Fig. 25. Tangerine, showing female ovipositing in
ripe fruit.

that developed in orange tissue was inadvertently lost, so that it is not definitely known whether or not adults emerged from this particular material.

The Mediterranean Sweet variety was tested in a limited manner. When the oranges were dissected one contained small larvae. Closer examination showed that a female had successfully placed her eggs in the orange to a depth adequate for hatching.

Tangerine.—Females were successful in ovipositing (fig. 25) in this loose-skin type of citrus fruit (*Citrus deliciosa*). The eggs were deposited beneath the skin, though not into the fleshy pulp tissue. While hatching occurred, larvae were not found in any of the fruits examined, and none reached maturity in these tests. Since eggs or small larvae were not artificially placed in the flesh tissue, it is not known whether or not larvae are capable of developing in this medium.

Grapefruit.—Both Marsh and Duncan grapefruit (*Citrus grandis*) were placed in cages with ovipositing females. The actions of the flies were similar to those reported for the Valencia orange. One fruit in par-

ticular exhibited eleven "brown spot" areas when removed, indicating
many attempts at oviposition (fig. 26). Hardness data (fig. 23) showed
that the skin of grapefruit was considerably softer than other species of
citrus tested; however, no eggs were deposited deep enough to insure
hatching.
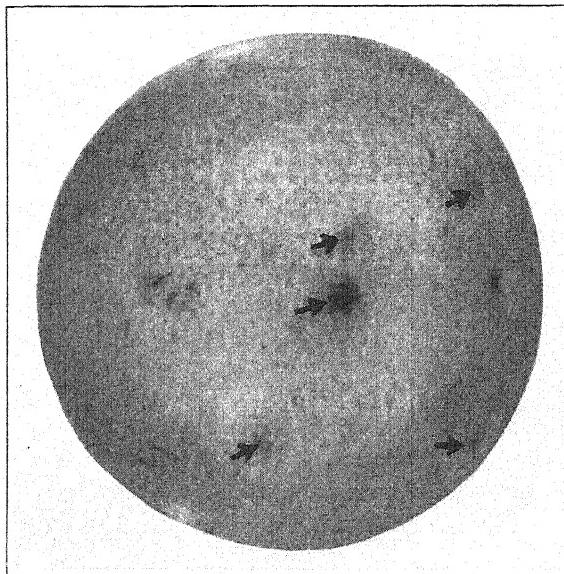
Fig. 26. Grapefruit, showing points where many unsuccess-
ful attempts at oviposition were made.

Lemon.—Eureka and Lisbon lemons (*Citrus limonia*) were both
tested under laboratory conditions. Females repeatedly attempted ovi-
position without success. In twelve lemons of each variety, eggs were
artificially inserted. The final check of these failed to reveal that any
larvae had matured; in fact none was found, though indications of lar-
val feeding were noted in several fruits.

Apple, Pear, and Quince.—The Jonathan and White Pearmain va-
rieties of apple (*Pyrus malus*), the Bartlett pear (*Pyrus communis*),
and the Orange quince (*Cydonia oblonga*) were used in laboratory tests.
Eggs were deposited in all these species of pome fruits and hatching
occurred; however, in no instance did larvae reach maturity. Flies have
been commonly observed on these fruit trees where they were growing
interplanted with infested Eureka walnut trees; however, neither eggs
nor larvae have been found in any of the fruits under such conditions.

Plum.—Most of the plums (*Prunus salicina*) growing in the infested
area are harvested before the peak of seasonal activity of the fly. How-

ever, a late-maturing strain of the Satsuma variety was tested. Of the twelve plums placed in cages with flies, eggs were deposited in five. These eggs hatched, but none of the larvae reached maturity.

Fig.—The Kadota fig (*Ficus carica*) was tested rather extensively. Many branches bearing figs in various stages of development were placed in cages with adults. The females attempted oviposition in many instances; however, no eggs were deposited. The milky, viscous exudation emanating whenever the skin is punctured possibly is repulsive to the fly. Females have been observed to withdraw their ovipositor almost immediately after the puncture is made and they apparently have considerable difficulty in cleaning it.

A cheesecloth cage (12 × 12 ×12 feet) was built over a small tree heavily laden with fruit, and a total of 500 flies was introduced at varying intervals. At the end of the season, no larvae or pupae were found.

Grape.—The Tokay, Muscat, and Emperor grapes (*Vitis vinifera*) were tested in the laboratory. In one instance a female was observed to attempt oviposition but no eggs were found in any of the grapes, and at the end of the experiment, no larvae or pupae were found.

Prickly Pear.—Females oviposited readily in the cactus fruits (*Opuntia* sp.). In these tests it was very apparent that the flies did not care to rest on the fruit since they were usually noted there only when ovipositing. On dissecting several fruits it was noted that the eggs had hatched, but none of the larvae reached maturity.

Pecan.—Green fruits of both seedling and budded pecans (*Hicoria olivaeformis*) were tested. Flies frequently attempted oviposition, but in no instance were they successful in penetrating the husk to deposit eggs.

Sierra Sweet Bay.—Sierra sweet bay (*Myrica hartwegii*) occurs commonly in the canyons of the Sierra Madre and has many of the attributes of the Juglandaceae—in fact the families are closely related. A limited number of the nutlets were placed in a cage with flies very late in the season. Oviposition was not observed, and neither larvae nor pupae were found at the end of the experiment.

Avocado.—Several Mexican varieties of avocados (*Persea gratissima*) that ripen in the early fall and a later variety, the Fuerte, were placed in cages with flies. Females were observed to attempt oviposition without success; the fruits, which were green, possessed a hard skin. Neither larvae nor pupae were found at the end of the experiment.

Pomegranate.—Ripe pomegranates (*Punica granatum*) were placed in cages with flies. Several attempts at oviposition were observed, though in no case were the females able to insert their ovipositors into the skin.

Potato.—The Idaho Russet potato (*Solanum tuberosum*) was used in these tests. In three instances eggs were deposited beneath the surface of the tuber, and a few eggs were placed on the surface near the punctures. Examination six weeks later showed that the eggs had hatched but only one larva was found. This particular larva was in the second
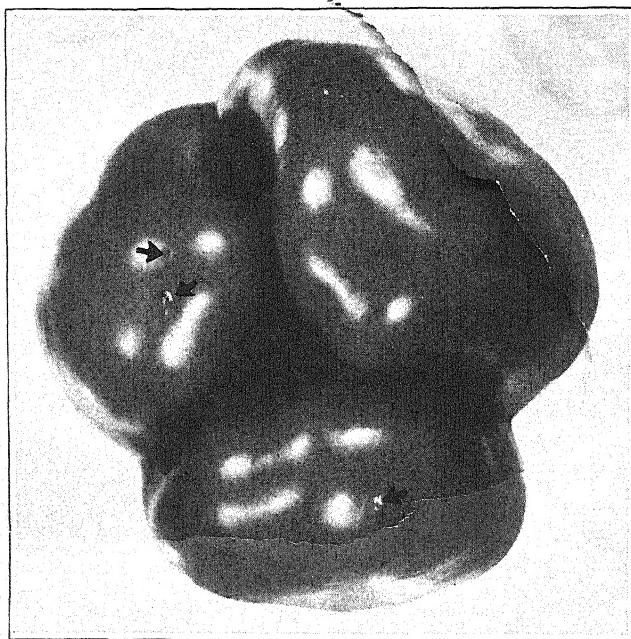
Fig. 27. Bell pepper: the arrow at the upper left indicates cavity of eggs deposited normally; other arrows indicate eggs on surface above cavities that are filled with eggs.

instar and died before maturing. This test was conducted in an effort to find a host which could be used for continuous rearing of the insect in the laboratory.

Eggplant.—Many instances were recorded of females ovipositing in the mature eggplant (*Solanum melongena*). One female died with the ovipositor inserted. The eggs hatched but none of the larvae reached maturity.

Bell Pepper.—The flies oviposited very freely in mature bell peppers (*Capsicum grossum*). There were several cavities of eggs in each pepper tested (fig. 27). In one instance a batch of nine eggs was found adhering to the surface of the pepper near a cavity containing eggs. None of the larvae reached maturity.

Tomato.—Ten ripe and twenty green tomatoes (*Lycopersicum esculentum*) were used in these tests. Eggs were deposited in all tomatoes used and in some cases several batches were found in each. When eggs were deposited in green tomatoes, a callus formed around and below the puncture in the tissue. The eggs hatched but most of the larvae died before maturing. However, two larvae reached maturity and formed normal-sized pupae. When adults failed to emerge the pupal cases were opened and the insects found to be dead. It is questionable whether or not mortality was due to environmental conditions after pupation or to the effect of the host during larval development.

Discussion of Studies on Possible Hosts.—While most of the host studies were preliminary in nature, they are indicative of the fact that the flies will attempt to deposit their eggs in many kinds of fruits under artificial conditions. In some instances degeneration of the fruit undoubtedly affected the development of the young larvae. Thus it appears that, given natural host conditions, some larvae would probably mature in certain of the fruits tested.

The insect has never been observed even attempting to oviposit in the stem of a plant, whether succulent or otherwise. Therefore, the larvae appear to be entirely restricted to inhabitation of fruits.

During the five years that the insect has been studied, most species of fruits and vegetables growing within the infested area have been rather carefully observed for indications of infestation. None has been noted to be infested, with the exception of walnut and peach.

The extent to which fruits other than the preferred host, walnut, will be attacked under natural conditions is problematical. However, the observations and experimental data to date indicate that the species is not likely to become of economic importance on other crops.

INJURY AND ECONOMIC IMPORTANCE

The degree of infestation is dependent upon a combination of factors and may vary from less than 1 per cent to over 95 per cent of the nuts on the tree.

Shell Stains.—Injury caused by *Rhagoletis completa* is manifested in several ways. The principal type is shell stain, resulting from the feeding of the larvae within the exocarp or green husk of the nut. The surface of the husk directly above the feeding tunnels assumes a black color caused by the decay that takes place beneath (fig. 28). This blackened area increases as the larvae extend their feeding range and in many cases includes the entire husk, though usually about one-half or less of it is affected. The juices from the soft decay of the inner husk permanently

darken the shell of the nut (fig. 29). This staining is probably caused by tannin released from the broken-down tissue, and in many instances it extends entirely through the shell.

In laboratory experiments, normal newly harvested walnuts were partially submerged in tannic acid solutions of varying concentrations and lengths of exposure. Characteristic staining of the submerged por-

Fig. 28. Characteristic external appearance of injury by larvae feeding within husk. Note that injured husk tissue has not shrunk, and that a definite line marks the color changes from black to normal green.

tions of the shells was produced with concentrations as low as 1 per cent when exposed for 48 hours. In an attempt to remove the stain from infested walnuts, experiments involving the use of chloroform, ether, sulfuric acid, chloride of lime, potassium permanganate, chlorine, and ethylene were conducted with negative results. Tests with sand blast were also carried out without success. Any nut having a shell stain which is not removed by the regular chlorine bleaching process in packing-house operations becomes a "cull."

Since there is no known method of removing the stain resulting from infestation by this insect, nuts so infested are classified as culls, and the returns to the producer are appreciably reduced as a result. Such cull walnuts are normally processed in a cracking plant and the carefully

graded meats marketed in bulk under trade names representing their quality. Packing-house records show that cull walnuts net the producer approximately 50 per cent less than normal walnuts, even though they contain unimpaired kernels. The Eureka variety, which is very susceptible to infestation, usually commands the highest market price.
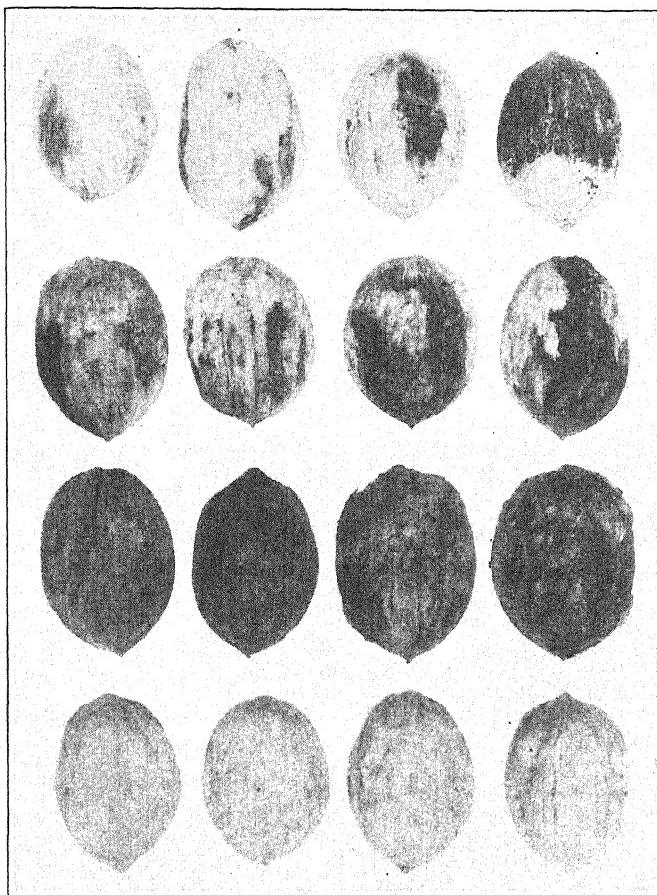
Fig. 29. Upper three rows show varying amounts of shell surface stained as a result of infestation. Lower row shows uninfested nuts.

All infested walnuts, however, do not become culls, and when they are not classed as culls it is doubtful whether or not an economic loss results. Usually from 10 to 25 per cent of the nuts attacked exhibit no evidence of having been infested after they are hulled at harvest. The extent of injury is somewhat dependent upon seasonal conditions with

reference to the development of the host and the emergence and subsequent development of the immature stages of the fly.

Reduction in Quality of Kernels.—The secondary type of injury includes a reduction in quality of some of the kernels. According to the


```
        INFESTED
Date    Nov. 28, 1931
     Variety  EUREKA
Lot B        Cracks #6
Light        20 per cent
Amber        35 per cent
Black        2 per cent
Sound        55 per cent
Moldy        15 per cent
Wormy        1 per cent
Shrivelled   15 per cent
Blank        8 per cent
Weight       537 grams
```

Fig. 30. Typical lot of 100 infested walnuts showing standard method of grading kernels in order to determine the extent of injury resulting from infestation.

"grades" established by the California Walnut Growers' Association, the value of walnuts is primarily dependent upon a clean, light-appearing shell, and such factors pertaining to the kernel as insect damage,

TABLE 1

EXTENT OF INJURY TO WALNUT MEATS DUE TO INFESTATION BY RHAGOLETIS COMPLETA, 1929–1931, AS SHOWN BY CRACK TESTS

Year and group*	Light	Amber	Black	Moldy	Shrivel and blanks	Sound	Weight	Merchant-able†	Grade after culling†	Returns to producer† (orchard run)
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
	per cent	per cent	per cent	per cent	per cent	per cent‡	grams	per cent		cents per pound
1929										
A Normal	85	6	0	0	9	91	565	94	Diamond	18.9
B Infested	72	13	1	5	9	85	555	86	Diamond	17.8
B compared with A	−13	+7	+1	+5	0	−6	−10	−8	....	−1.1
1930										
A Normal	38	28	1	0	33	66	459	73	Emerald	13.1
B Infested	40	25	1	1	33	65	455	74	Emerald	13.3
B compared with A	+2	−3	0	+1	0	−1	−4	+1	....	+0.2
1931										
A Normal	44	38	1	2	15	82	540	89	Emerald	13.6
B Infested	16	42	6	13	23	58	522	64	Suntand	9.3
B compared with A	−28	+4	+5	+11	+8	−24	−18	−25	....	−4.3
Average, 3-year period										
A Normal	56	24	1	1	19	80	521	85	....	15.2
B Infested	43	27	3	6	22	69	511	75	....	13.5
B compared with A	−13	+3	+2	+5	+3	−11	−10	−10	....	−1.7

* Each group consisted of 10 lots of 100 walnuts each.
† Data calculated by A. W. Christie, Field Manager, California Walnut Growers' Association, from data supplied in other portions of this table, together with available packing-house records.
‡ Columns 1+2+3+4+5=100; columns 6+3+4+5=100.

color, shriveled condition, and presence of mold. Larvae feeding within
the green husk apparently affect the kernel with respect to certain of
these factors, particularly in those nuts attacked earlier in the season.
Since the juice that causes the stain usually penetrates the entire thick-
ness of the shell it probably darkens the meat in some instances. Be-
cause the largest percentage of eggs is deposited in the stem region of
the husk, the larvae first attack the tissue in that region. Consequently
some of the conductive tissue transporting nutritive substances to the
developing kernel is destroyed. It seems probable that under certain
extreme conditions shriveling of the kernel results. Humidity condi-
tions conducive to the growth of fungi on the kernels probably exist as
a result of infestation, particularly when larvae have tunneled the en-
tire husk and the ensuing damp, mushy decay is present. Thus when
accompanied by protracted climatic conditions favorable to fungi devel-
opment, mold is likely to become evident on the kernels.

Crack tests were made to ascertain the effect of infestation on the
quality of the kernels (fig. 30). In order to obtain a significant com-
parison, random samples of 1,000 nuts exhibiting evidence of having
been infested, and another of noninfested nuts, were taken from the
same grove at harvest. These samples were dehydrated and otherwise
handled under identical conditions. The results of these crack tests are
presented in table 1.

When the various factors affecting quality of the walnuts in normal
and infested lots are averaged for the three-year period, it is evident
from the data in table 1 that color of kernel, moldiness, proportion of
blanks, and weight are appreciably affected by infestation. The data
show an average reduction in merchantable kernels of 10.5 per cent,
with the resultant reduction of 11.5 per cent in net returns to the pro-
ducer. The loss ranges from 0 to 25 per cent. The data given with respect
to percentage of merchantable nuts and cents per pound net to producer
(orchard run) were calculated from the crack-test records, and the in-
jury resulting from stained shells in the infested lots was ignored. By
treating the data in this manner, it is possible to arrive at the reduction
in value of the walnuts due to infestation, aside from the primary type
of injury. Therefore, by closely estimating and calculating, the total re-
duction in net returns to the producer from injured nuts amounts to
from 50 to 75 per cent, allowing 50 per cent for the primary type of in-
jury and from 0 to 25 per cent for the secondary type.

Harvesting Costs.—Harvesting costs are increased somewhat by the
presence of infested walnuts. The husks of the majority of the infested
nuts do not split normally and allow the nut to drop (fig. 31). Thus
more time is consumed at harvest in shaking the tree to remove these

"sticktight" walnuts. After these infested walnuts are on the ground, the adhering husks must be removed by hand labor or by hulling machinery. Furthermore when freshly hulled, infested walnuts are put into sacks or piles with uninfested nuts, some staining of the shells of the latter occurs.

Fig. 31. Upper row, "sticktights" produced by infestation. Lower row, uninfested walnuts, showing how husk normally splits.

Incidental Expenses.—Other economic considerations are the costs incident to enforcement of quarantines for the prevention of artificial spread. This is particularly important since approximately 30 per cent (30,000 acres) of the total walnut acreage in California is planted to susceptible varieties, most of which is not within the present infested area.

LIFE HISTORY AND HABITS

The treatment of life history and habits in this study deals with each stage of the insect in the sequence of natural occurrence. To conserve space many of the detailed data are presented graphically only. Therefore the points in graphs were merely connected and no attempt was made to smooth curves. In many instances several sets of data are plotted on the same chart, thus precluding the inclusion of the smoothed curve together with that connecting the points.

ADULT

Cages Used in Studying Adult.—During the early part of this study considerable difficulty was experienced in keeping adults alive for appreciable lengths of time in any type of container or cage other than inverted jelly glasses. Most of the workers on other species of *Rhagoletis* have reported similar experiences. The inverted jelly-glass type
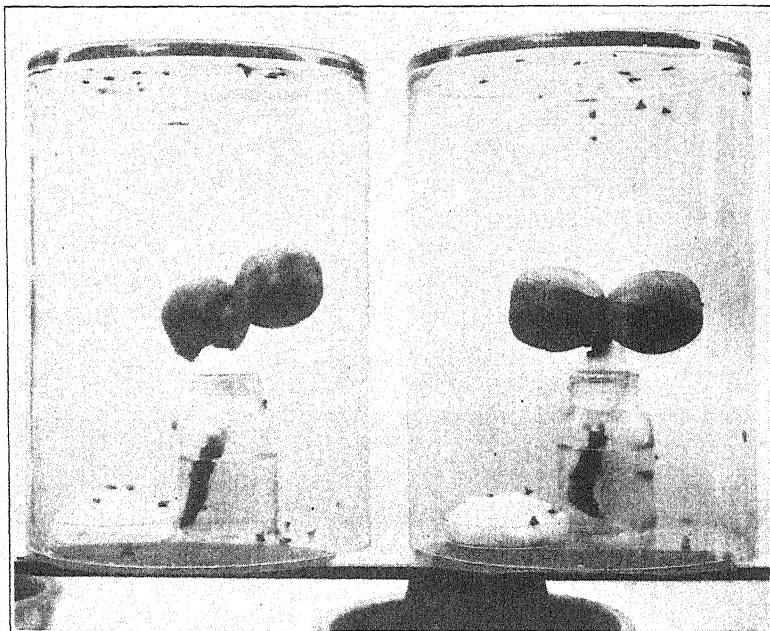
Fig. 32. Inverted battery-jar cage and typical set-up used in biological studies. The section of petri dish contains absorbent cotton, saturated with liquid food. Jar extends beyond the edge of the plate-glass shelf, to permit aeration. The vent thus provided is covered with wire screen.

of cage was decidedly too small to carry on the desired studies. The success of such a cage appeared to be due to the maintenance of a humidity condition bordering on the optimum. It seemed evident therefore, that any type of cage that would duplicate the conditions of the inverted jelly glass would be satisfactory. Preliminary tests demonstrated the adaptability of inverted battery jars for these studies.

A description of the procedure employed throughout most of these studies follows: Battery jars 9 inches in height and 7¾ inches in diameter, with ground edge, were inverted on sections of plate glass ¼ inch thick, 9 inches wide, and 5 feet long (fig. 32). Sections of this length

were easily handled and kept clean. It was necessary to mount the plate glass on pedestals in crocks of water, thereby isolating the section and avoiding invasion by ants. One half of a 2½ inch petri dish, containing a wad of absorbent cotton thoroughly saturated with a 25 per cent sucrose solution, was placed in the cage to supply food and moisture. The inverted jar was left near enough to the edge of the plate glass to permit an opening of approximately ½ inch at the widest point.

The flies to occupy the cage were drawn into the glass tube of a regular suction-type catcher and then discharged into the inverted jar. This type of insect catcher was used exclusively for such handling of flies to minimize injury. When the desired number of flies had been injected
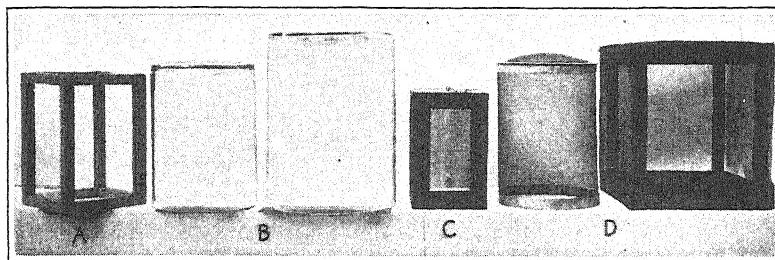
Fig. 33. Types of cages used in biological studies: *A*, quartz glass; *B*, flint-glass battery jars; *C*, 16-mesh wire-screen cage for suspending in foliage of trees; *D*, 16-mesh wire-screen cages used in laboratory.

into the cage, a small piece of 16-mesh screen was placed under the edge of the jar to prevent their escape. The weight of the jar held the screen in place. The vent prevented condensation of moisture on the sides of the jar during the day. Droplets of moisture were usually present on the walls of the jar during the night, but these in no way interfered since the flies did not move about to any appreciable extent after dark. In all cases, except in experiments where it was impracticable, such as the toxicity series, the jars and plate glass were washed once a week. This proved to be important in keeping flies apparantly normal over periods of weeks. A jar of the size indicated will accommodate about 200 flies without undue crowding. It is believed that this type of cage was fairly satisfactory for laboratory handling of the flies with the possible exception of the effect of certain rays of the sun's spectrum. While there is practically no information available pertaining to this matter with regard to insects, it seems probable that beneficial light rays would be excluded by the type of glass from which the battery jars are manufactured. Limited studies were made with quartz-glass cages.

Other types of small cages were tried but none was satisfactory (fig. 33). Cages one foot square, completely covered with 16-mesh wire screen,

and cages with one half covered with wire screen and the remainder with cheesecloth, were tested extensively. In some instances improvised humidifiers, consisting of wicks or absorbent cotton in bottles of water, were used. The life of the flies was prolonged when the humidity was artificially increased; but still they did not behave normally. In only a few instances out of some 50 cages set up in this manner was oviposition obtained.

Of the larger types of cages over small trees the cheesecloth-covered cage was satisfactory, while the one covered with 16-mesh wire screen was not. Two walnut trees of the same variety were covered with 12 × 12 × 12 foot wire-screen cages, while two more trees were covered with cheesecloth cages of the same size. These cages were stocked with flies and otherwise managed under as nearly comparable conditions as possible. Flies remained alive over the entire season in the cheesecloth cages and infested 100 per cent of the nuts, while in the screen cages the flies were all dead at the end of four weeks, and most of them at the end of three weeks. The screen cages were restocked after four weeks. The degree of infestation of nuts was less than 50 per cent at the end of the season. It is interesting to speculate on the explanation for the difference in environment apparently created by the two types of cages. The humidity factor readily suggests itself, in view of the experience with smaller cages.

Emergence from the Soil.—In freeing itself from the pupal case in the soil to emerge, the adult pushes off the anterior end of the case. The point of rupture of the puparium is at the circular cleavage line in the middle of the first abdominal segment. Occasionally there is a rupture along the horizontal cleavage line also. The ptilinum no doubt plays an important rôle in forcing this pupal cap off. When the fly leaves the puparium the entire body is of a light color, very plastic, and capable of being greatly distorted. This faculty, together with the aid of the ptilinum, enables the fly to work its way through small cracks and other small places. As an example in many instances dead bodies of newly emerged flies have been found in the center of tightly rolled cotton plugs in glass vials in which pupae were being temporarily held. After the surface of the soil has been gained, the fly very diligently cleans itself. The ptilinum is inflated and deflated in the cleaning process, while being vigorously stroked with the forelegs which also serve to clean the antennae and mouth parts. On emergence the wings are folded along the longitudinal veins. The fly walks briskly about but stops frequently to stroke the much-folded wings and the abdomen with the hind legs. The three pairs of legs are cleaned by rubbing them together. In a total of over 50 flies observed emerging at different times, an average period of

20 to 30 minutes was necessary for the wings to become extended and hardened sufficiently for flight. The natural color pigments of the insect become conspicuous after several hours' exposure to daylight.

In connection with control experiments, detailed information regarding emergence was necessary. Therefore, the emergence studies were fairly extensive and extended from 1928 to 1932 inclusive.

Methods Used in Emergence Studies.—The cages used in these studies were 7 feet long, 6 feet wide, and 3 feet high, and were covered with
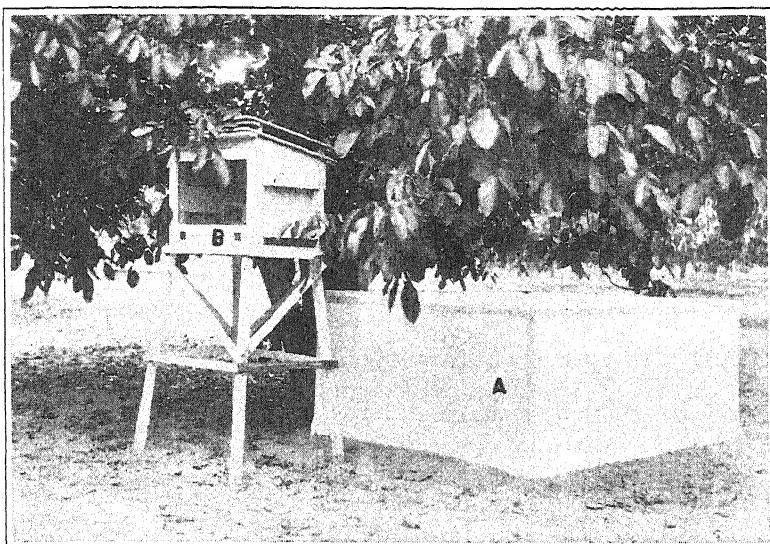
Fig. 34. *A*, standard type of adult soil-emergence cage (7 × 6 × 3 feet) in typical location under walnut tree. *B*, shelter containing thermograph for recording soil temperature within emergence cage.

cheesecloth (fig. 34). They were placed under infested trees, centered on the tree trunk, and extended parallel with the irrigation furrows. An effort was made to locate them at points representative of the particular area with reference to soil type, size of trees, and other factors. Since large numbers of flies were needed for other studies, the population of certain cages was augmented artificially in the following manner: A frame 6 feet long and 5 feet wide, with ¾-mesh chicken wire bottom, and mounted on legs 1 foot high, was placed over the site of an emergence cage. Infested walnuts were put into this frame and the larvae came out of the husks to enter the soil normally.

The cages were erected and covered with 50-mesh cheesecloth in June each year. This was early enough to catch the first emerging flies. An effort was made to ascertain the extent to which the cheesecloth cover-

ing altered the natural environment with respect to temperature and humidity. A hygrothermograph in an approved shelter was set up in a cage about one foot above the surface of the soil. This instrument had been carefully calibrated with the one to be set up under normal grove conditions. Furthermore, both instruments were checked by sling psychrometer readings at frequent intervals. Records from the emergence cage were kept for a period of three weeks. The comparison of conditions for a typical week is shown in figure 35.

The data show that in the emergence cages the temperature averaged approximately 1 degree higher at the upper limits and approximately
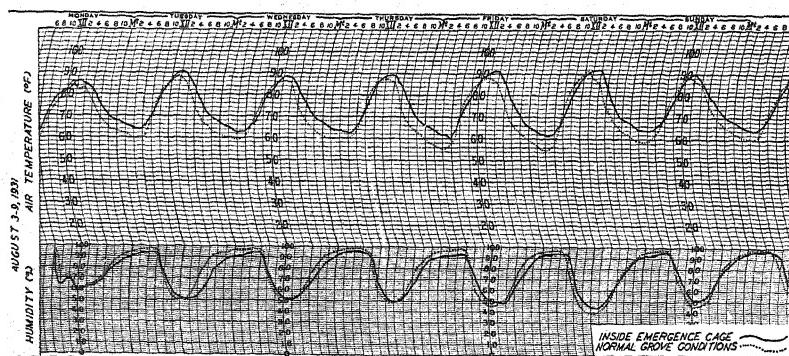
Fig. 35. Temperature and relative-humidity conditions existing inside an emergence cage in comparison with normal orchard conditions. (Typical 7-day period.)

2 degrees higher at the lower limits. The relative humidity varied inversely with the temperature to the extent of approximately 1 and 2 per cent, respectively. It seems improbable that the indicated differences in temperature and humidity between cheesecloth emergence cages and normal grove conditions influenced the emergence of adults.

Beginning with the emergence of the first fly of the season, each cage was visited daily at approximately the same time. The flies were carefully collected and a daily record was kept of total number of flies per cage with the ratio of females to males. Many different types of containers for collecting were tested in an effort to find the most satisfactory one from the viewpoint of speed in collecting, minimum injury, and ease of segregating and counting. A test tube, 15 mm in diameter, was found to be best adapted for use. Since the flies were usually on the walls and ceiling of the cage, the tube could be placed over a single fly very readily after a little practice. The insect usually landed in the bottom of the tube after the effort made to escape when the tube was placed over

it. However, the flies generally climbed upward on the walls as soon as equilibrium was gained, which made it necessary to jar the tube often by giving it a sharp rap against the palm of the hand. Fifteen to twenty flies were usually collected in one tube, which was then plugged with cotton and placed in the collecting box. When the box with tubes and flies was kept in the shade, the flies usually quieted down and by the time all were collected from the cage the ones taken earlier could be readily segregated and counted. The females were readily singled out because of the somewhat pointed abdomen.

The data pertaining to emergence each season are graphically presented and discussed, while a summary of the total emergence data concludes the treatment of this phase of the study.

Definition of Terms Used in Emergence Studies.—In discussing emergence several of the terms used require definition in order that the author's conception may be clearly understood. "Second generation" refers to those individuals that may emerge from the soil during the season in which they pupate. "Annual generation" refers to those individuals that remain in the soil throughout one winter, adults emerging the year after the season when larvae entered the soil and pupated. "Biennial generation" refers to those individuals that emerge the second year after pupation. "Multi-annual generation" refers to those individuals that remain in the soil longer than one year. "Seasonal peak" of emergence refers to the time when the greatest numbers of flies emerged. "Median" of emergence refers to the time when 50 per cent of the total number of flies have emerged.

Emergence in 1928.—The emergence data for 1928 were limited, since only four cages were employed. These were located in a grove that was moderately infested in 1927. There was considerable variation in the rate of emergence in the different cages. However, the variation among the four cages, two seeded and two natural, was not greater than the variation within either one of these groups. A total of 1,459 flies emerged; the sex ratio was 53 ♀ to 48 ♂. A composite of the data obtained is presented in figure 36.

Emergence began on July 12 and gradually increased until the seasonal peak was reached on August 18. Then a gradual decrease took place until the end of the season. The last flies were collected in the cages on September 25. Thus flies were emerging daily during a period of 75 days. The curve representing seasonal emergence may be considered a normal frequency polygon, which is the expected type, considering the nature of the phenomenon. The daily emergence record shows that females emerged in greater numbers than did males during the

fore part of the season, while the reverse was the case during the latter
portion. Complete temperature records were not available during this
season.

A cage of carefully sifted soil was maintained, which contained ap-
proximately 4,000 pupae of the earliest-maturing 1928 larvae. No flies
emerged in this cage, indicating the absence of even a partial second
generation.
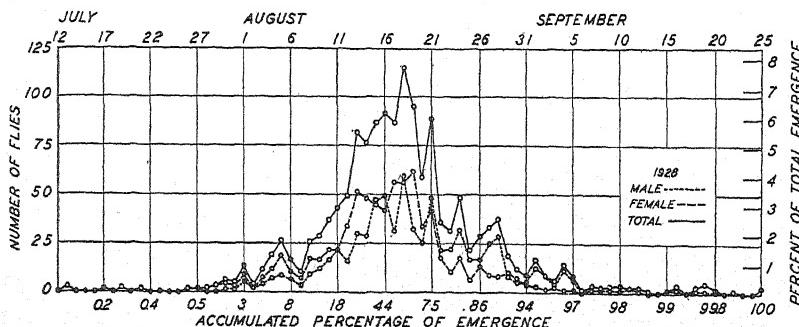
Fig. 36. Emergence of *Rhagoletis completa* in 1928.

Emergence in 1929.—A total of 20 cages supplied emergence data for
the season of 1929. These cages were distributed over the infested area
and were located in groves where a considerable degree of infestation
existed in 1928. Hygrothermograph records furnished complete data
regarding air temperature and humidity throughout the season. A total
of 7,398 flies emerged. The sex ratio was 53 ♀ to 47 ♂. The general emer-
gence data are shown in a composite chart, together with air temperature
and humidity records, in figure 37.

Emergence began on July 19, reached the seasonal peak on August 26,
and terminated October 9. Thus the total period of emergence was 82
days. The general shape of the emergence curve is similar to that of
1928. Likewise as in 1928, females were predominant in numbers during
the fore part of the season, with the situation becoming reversed during
the latter portion.

The mean daily air temperature and relative humidity shown in fig-
ure 37 represent an arithmetical mean of the readings at 2-hour inter-
vals. Relative humidity apparently has no bearing upon emergence and
is included on this chart only for reference convenience in dealing with
other phases of the study. However, there is an indication of a relation
existing between air temperature and emergence. Soil temperature is
directly related to air temperature.

From the beginning of the emergence period until the seasonal peak
is reached, the mean air temperature based on 5-day averages forms a

fairly smooth curve which corresponds to the curve of emergence of the flies. Soil temperature does not fluctuate so greatly nor so rapidly as air temperature; therefore it is unlikely that the several short periods of
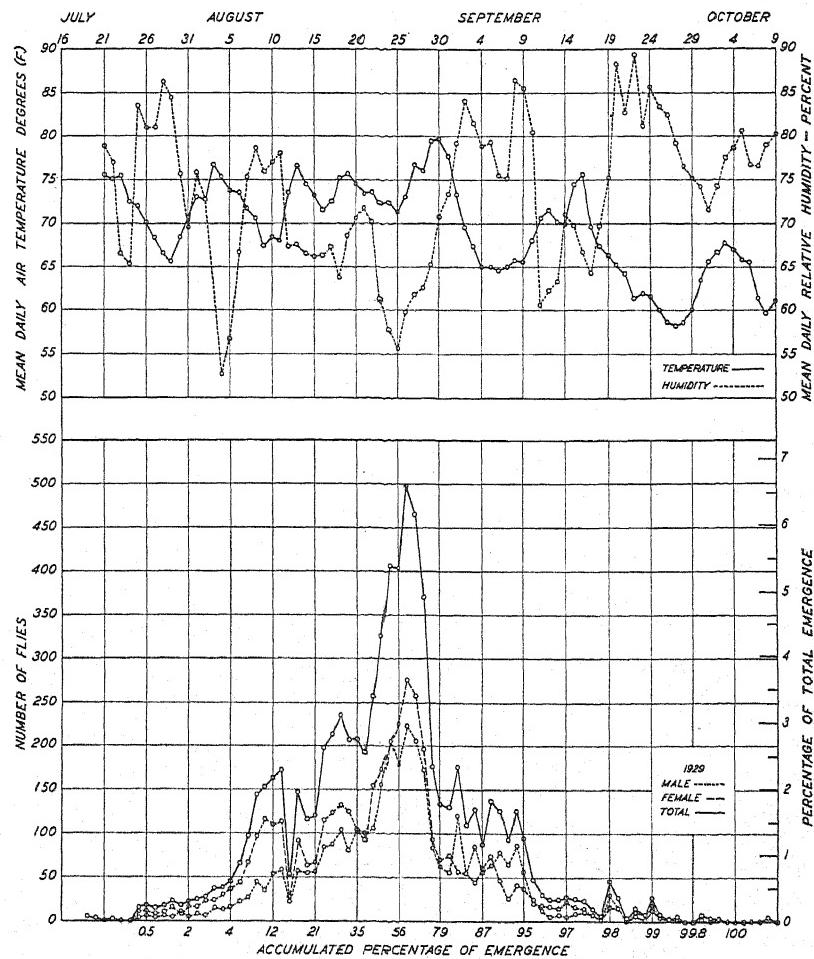
Fig. 37. Emergence of *Rhagoletis completa* in 1929, with air temperature and relative humidity.

lower air temperature just before the seasonal peak of emergence materially altered the temperature at a depth of several inches.

Data regarding time and rate of emergence of annual-generation flies in comparison with the biennial generation were obtained and are presented in figure 38.

A greater percentage of biennial-generation flies than of the annual-generation ones emerged early in the season. The accumulated emergence on August 23 was 94 per cent biennial in contrast to 43 per cent annual.
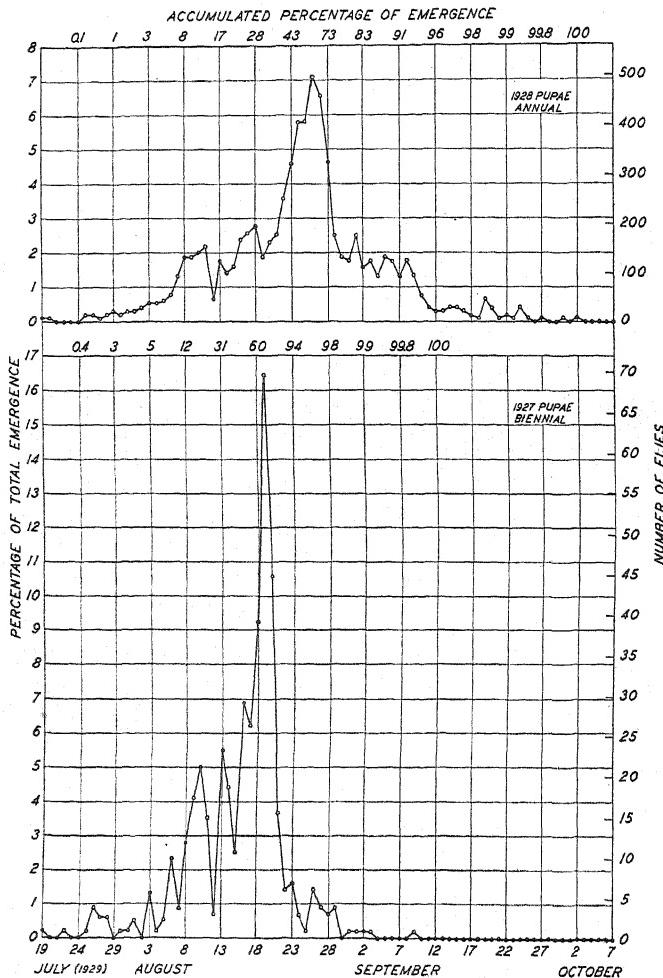
Fig. 38. Comparison of emergence of annual and biennial generations of *Rhagoletis completa* in 1929.

Emergence in 1930.—The emergence data for 1930 were obtained from 15 cages distributed in the infested area. A total of 8,965 flies emerged. The sex raio was 48 ♀ to 52 ♂. Throughout the period of emergence, thermographic records of soil temperature and hygrothermographic records of air temperature and humidity were maintained. The
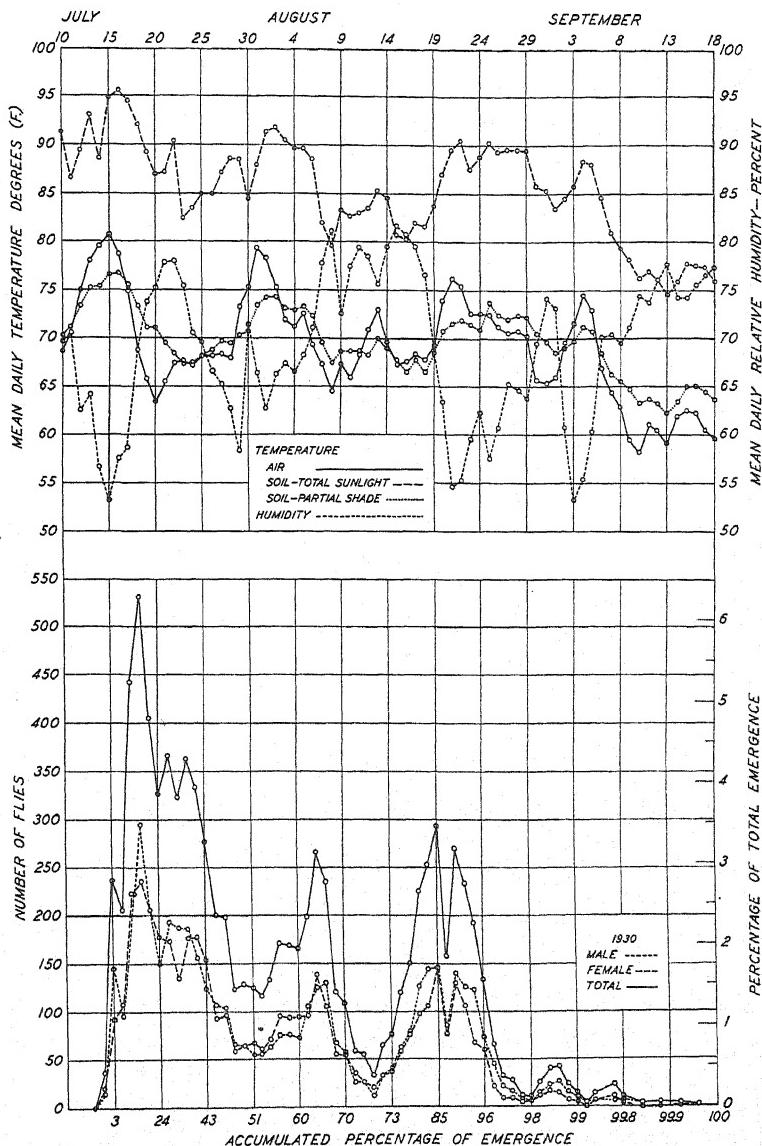
Fig. 39. Emergence of *Rhagoletis completa* in 1930, with prevailing soil temperature, air temperature, and relative humidity.

thermographs used were carefully calibrated at the beginning and were frequently checked throughout the season. The temperature of the soil at a depth of 3 inches in cages in total sunlight was compared with that of the soil in cages in partial shade at the same depth.

The data regarding fly emergence and records on air and soil temperature and relative humidity are presented in figure 39.

Emergence began on July 13, reached an early seasonal peak on July 18, and terminated on September 16. The total period was 65 days.

The records show only slight differences in the relative abundance of males and females throughout the emergence period. The general shape of the emergence curve differs greatly from those of the previous two years in that it is distinctly multimodal. This probably indicates the operation of certain factors affecting emergence heretofore not encoun-
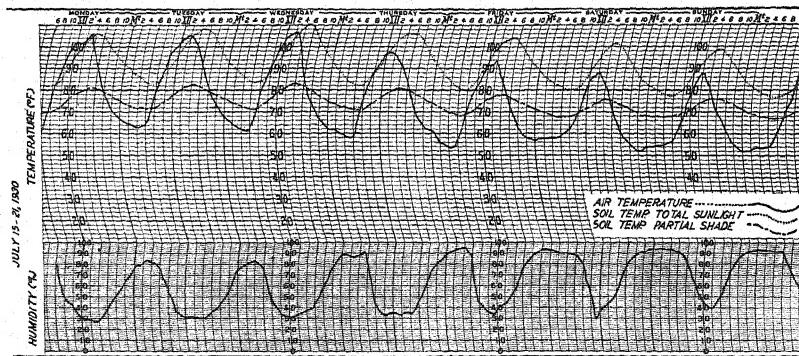
Fig. 40. Comparison of air temperature and soil temperature in cages in total sunlight, with soil temperature in cages in partial shade. (Typical 7-day period.)

tered, in which connection temperature is logically suspected. The soil-temperature data show a direct relation to emergence, since each major peak is closely followed by a major peak of fly emergence. The temperature of the soil in cages in total sunlight fluctuates only about one-half as much as air temperature. Soil temperature in cages in partial shade fluctuates slightly less than that in total sunlight. A lag of 2 or 3 days exists between important fluctuations in air temperature and the responding soil temperature. The records from charts of the three instruments for a typical week were transferred unaltered to one chart for comparative purposes. These data are presented in figure 40.

Data obtained regarding time and rate of emergence of annual-generation flies in comparison with the biennial generation are presented in figure 41.

Emergence of annual-generation flies began and reached the seasonal peak a few days earlier than in the biennial generation. However, after the first ten days of emergence the numbers issuing in both instances were fairly uniform. Minor seasonal peaks generally correspond in both cases.

Two cages containing approximately 3,000 pupae each, of the early-maturing 1930 larvae, were used to determine whether or not a partial second generation would develop. No flies emerged in either of the cages.

Emergence in 1931.—Twelve emergence cages were operated in 1931. A total of 11,856 flies emerged. The sex ratio was 47 ♀ to 53 ♂. The data obtained are presented in a composite chart in figure 42.
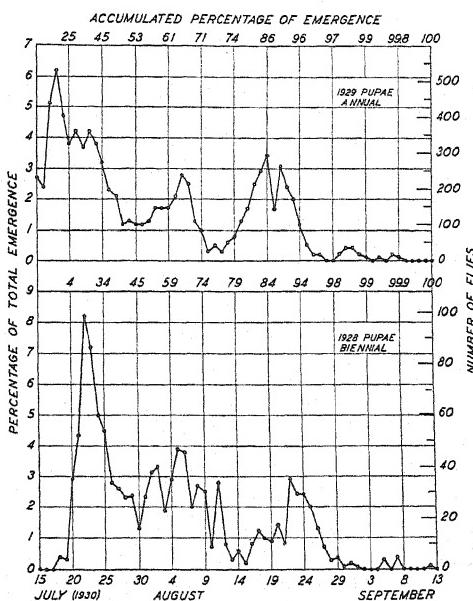
Fig. 41. Comparison of emergence of annual and biennial generations of *Rhagoletis completa* in 1930.

Emergence began on July 7, reached a very early seasonal peak on July 10, and terminated September 15. The total period of emergence was 70 days. Females and males emerged in about equal numbers throughout the emergence period. The general shape of the emergence curve is similar to that for 1930; therefore it likewise differs greatly from that for 1928 and 1929. The multimodal effect is apparently the result of the operation of factors similar to those that produced this condition in 1930.

Emergence began more abruptly in 1931 than in any preceding season. The mean daily temperature for the 10-day period prior to the beginning of emergence ranged from 75 to 79 degrees. Since these particular temperature data are not shown on the chart, the relation of temperature to the seasonal peak of emergence is not discernible. Sec-
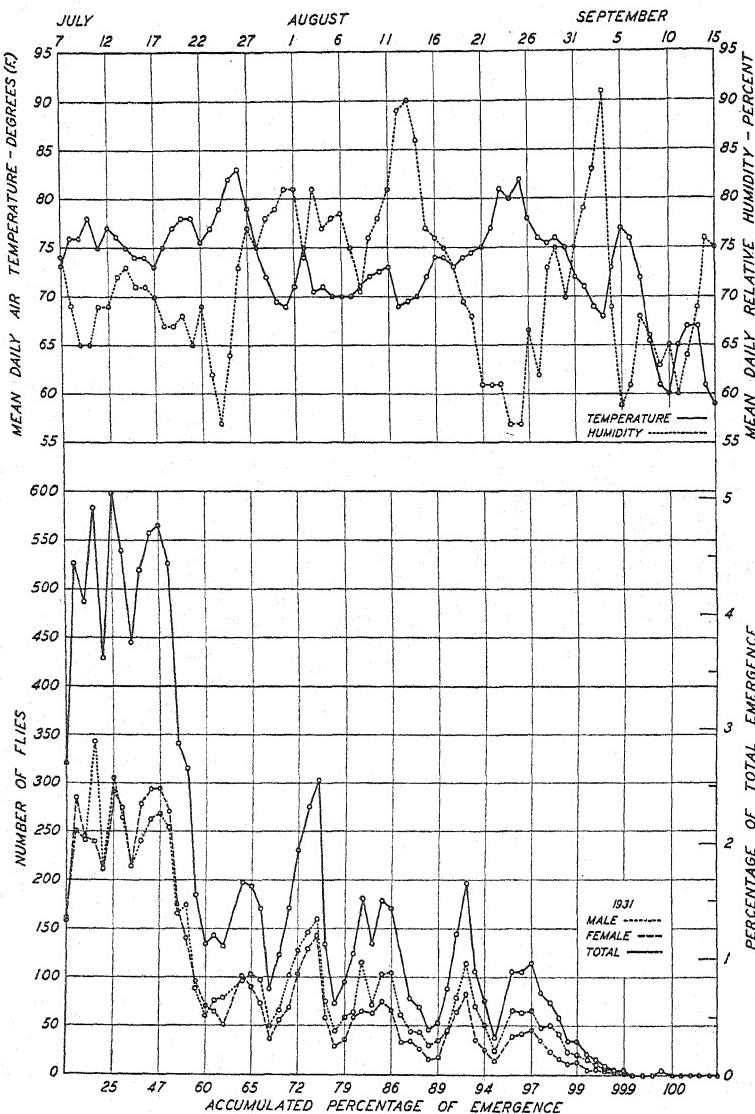
Fig. 42. Emergence of *Rhagoletis completa* in 1931, with air temperature and humidity.

ondary peaks of emergence rather closely follow rising temperatures above 70° F, indicating the relation of temperature to emergence.

Data obtained regarding time and rate of emergence of annual-generation flies in comparison with biennial-generation flies are presented in figure 43. A much greater percentage of biennial-generation flies

than of the annual-generation ones emerged early. In the former group the accumulated emergence on August 17 was 82 per cent, while in the latter it was 34 per cent.
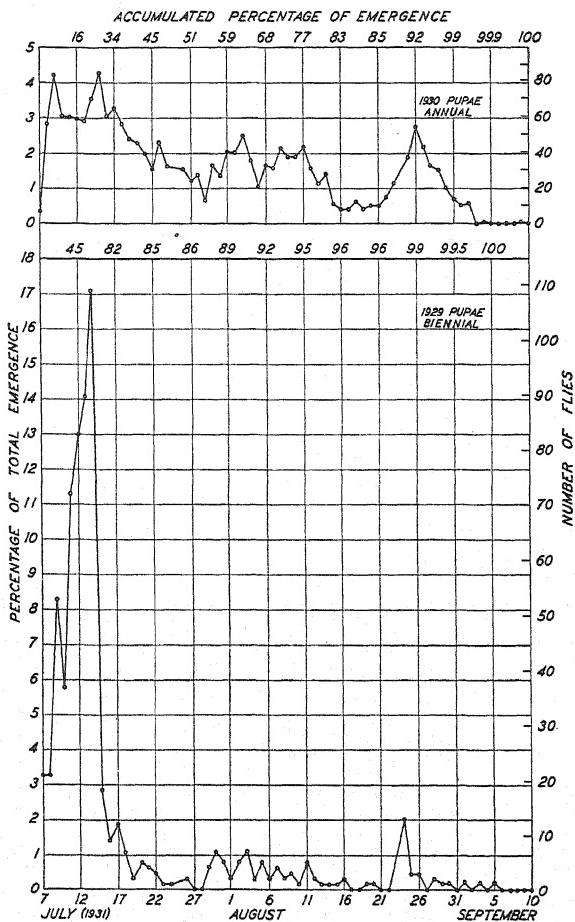
Fig. 43. Comparison of emergence of annual and biennial generations of *Rhagoletis completa* in 1931.

The time and rate of emergence from cages located on the north, south, and west exposures of trees were compared in 1931. These data are presented in figure 44. A material difference exists in the emergence of flies in the variously located cages. The emergence was most rapid on the west exposure, followed by the south and north. These differences are probably related to temperature.

Emergence in 1932.—Fifteen emergence cages were used in 1932. A total of 7,400 flies emerged. The sex ratio was 50 ♀ to 50 ♂. The data obtained are presented in figure 45.

Emergence began on June 29, gradually increased until August 10 when the seasonal peak was reached, and terminated on September 24.
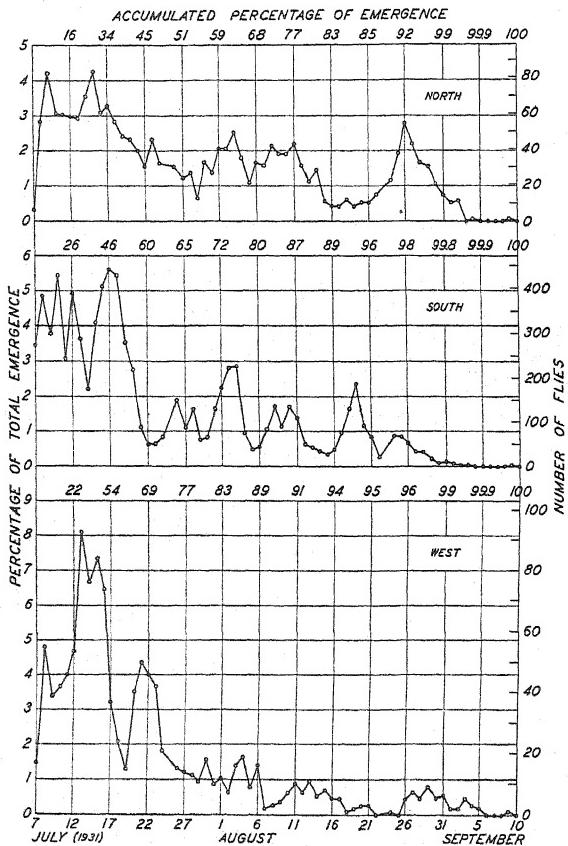
Fig. 44. Comparison of emergence of *Rhagoletis completa* in cages located on north, south, and west exposures of trees in 1931.

The total period of emergence was 96 days. Females were more abundant than males during the fore part of the season, while the situation was reversed during the latter portion.

The general shape of the emergence curve for this season more nearly approaches the normal frequency type than do those of 1930 and 1931 and in this respect is similar to that for 1928 and 1929. The relation of temperature to emergence is evident. The mean temperature averaged by 5-day periods during the fore part of the season increased gradually

and was accompanied by increased emergence. Just before the seasonal peak of emergence was reached, the temperature dropped; however, the soil temperature apparently remained sufficiently high to cause emergence to continue at a high rate for several days. The rapid drop in temperature probably produced the bimodal effect shown by the emergence curve.
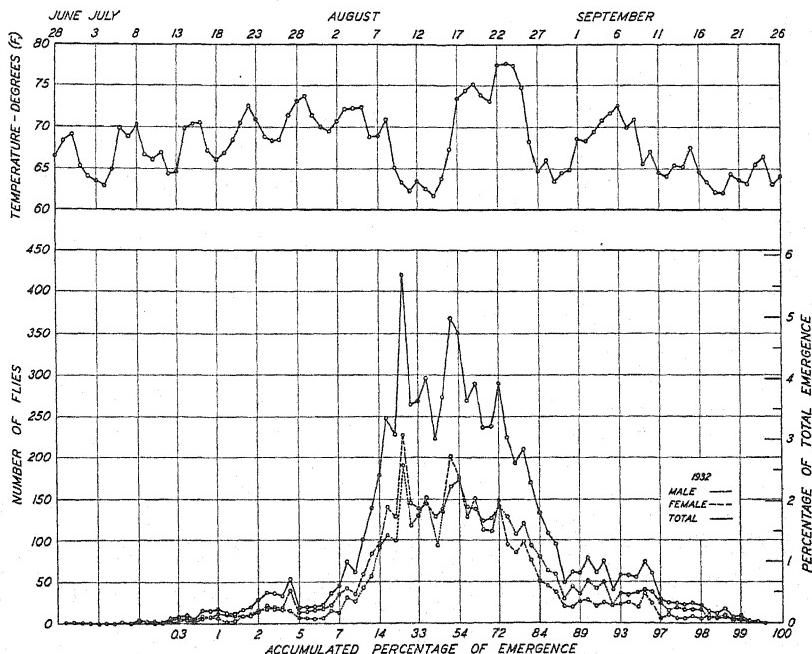
Fig. 45. Emergence of *Rhagoletis completa* in 1932, with air temperature.

Data obtained regarding time and rate of emergence of annual-generation flies and biennial-generation flies are presented in figure 46. A considerably greater percentage of biennial-generation flies than of annual-generation ones emerged early in the season. In the former group the accumulated emergence on August 7 was 71 per cent, in contrast to 25 per cent in the latter group.

Data regarding emergence from cages located on the north and south exposures of trees were obtained in 1932 and are presented in figure 47. Emergence began earlier in the season and the rate was more rapid in the southern cages than in the northern ones. The accumulated emergence on August 12 in the former was 37 per cent, while in the latter it was 13 per cent. The indicated differences are in accord with the 1931 data.
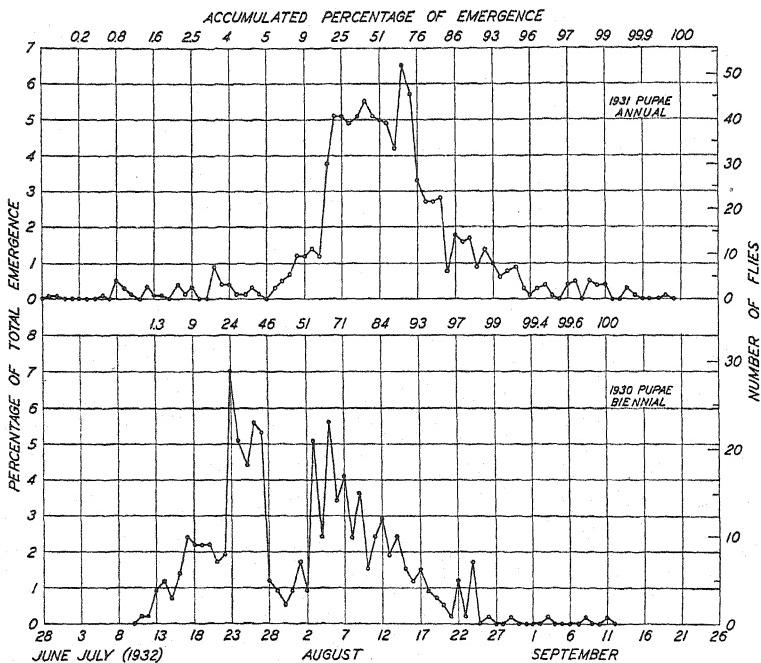
Fig. 46. Comparison of emergence of annual and biennial generations of *Rhagoletis completa* in 1932.
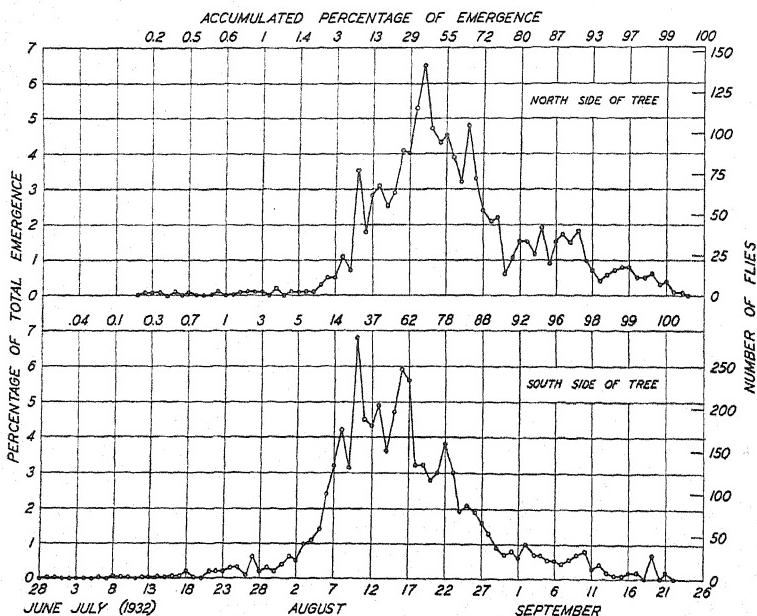
Fig. 47. Comparison of emergence of *Rhagoletis completa* in cages located on the north and south exposures of trees in 1932.

The effect of depth of pupae in the soil upon time and rate of emergence was obtained during 1932. In 1931, pupae were buried at a 3-inch depth in certain cages and at a 12-inch depth in others, in soil that had been freed of pupae by sifting. Emergence records obtained from these cages are presented in figure 48. The time and rate of emergence were materially increased in the cage 3 inches in depth over the cage 12 inches
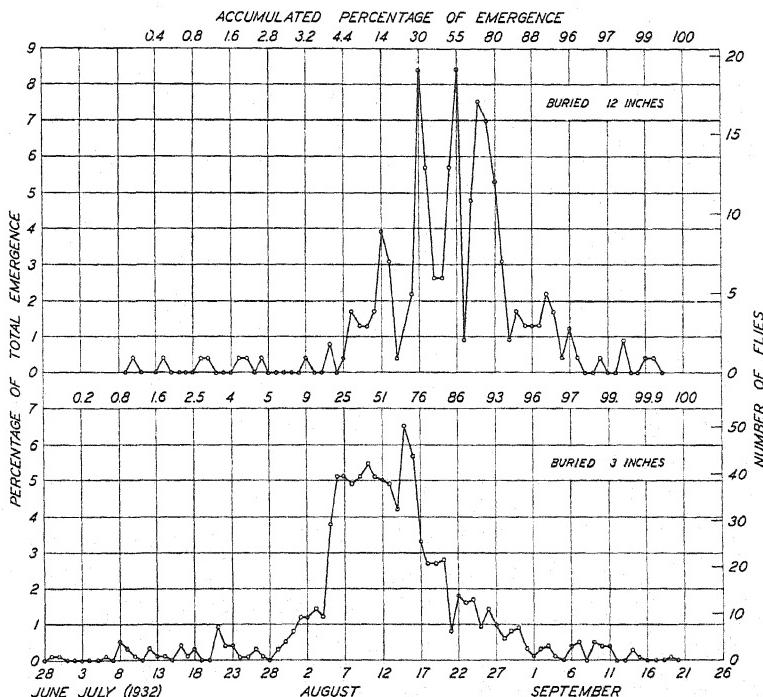
Fig. 48. Comparison of emergence of *Rhagoletis completa* from 3-inch and 12-inch depths in the soil in 1932.

in depth. The accumulated emergence in the former on August 12 was 51 per cent, while in the latter it was 14 per cent. Furthermore, emergence was considerably more erratic in the latter .

Mechanical Effect of Depth in Soil Upon Emergence.—The depth at which pupae are located in the soil is related to emergence. The temperature relations to emergence resulting from depth in soil have been briefly discussed. Limited information regarding the mechanical relation was obtained. The following experiments were conducted to determine the depth from which flies are capable of emerging in normally packed soil and in unpacked or relatively loose soil. Early in the season 200 live puparia were placed in soil in the bottom of each of ten 16-mesh

galvanized-wire-screen cages. These cages were 7 inches in diameter and the walls and bottoms were lined with wax paper to keep the emerging adults within the confines of the cage. All soil used was taken from the infested grove in which the experiment was conducted, and was sifted before being placed in the cages. In series *A* the soil was packed in a uniform manner to simulate orchard conditions, while in series *B* it was merely put into the cages without any effort to pack it. One *A* cage and

TABLE 2

DEPTH FROM WHICH ADULTS MAY EMERGE IN PACKED AND UNPACKED SOIL

Cage No.	Depth of puparia, in inches	Total number puparia buried	Empty puparia		Number live pupae at end of season	Number puparia un-accounted for	Flies collected	
			Number	Per cent of total			Number	Per cent of total that emerged*
Packed soil								
A1	8	200	137	69	40	23	26	19
A2	14	200	131	66	41	28	20	15
A3	20	200	122	61	60	18	21	17
A4	26	200	128	64	43	29	13	10
A5	34	200	61	31	52	87	1	2
Unpacked soil								
B1	8	200	135	68	36	29	48	36
B2	14	200	132	66	31	37	10	8
B3	20	200	115	58	38	47	72	63
B4	26	200	131	66	49	20	44	34
B5	34	200	98	49	50	52	20	20

* As indicated by empty puparia.

one *B* cage were placed at each of five depths in soil in a walnut grove so that the pupae were 8, 14, 20, 26, and 34 inches below the surface. Adults emerging on the surface in these cages during the season were collected at regular intervals. At the end of the season the soil in each cage was carefully sifted to obtain the empty pupal cases and the puparia from which no emergence had taken place. The results of these experiments are presented in table 2.

The data show that packing the soil materially affected the ability of emerging flies to reach the surface from all depths. It is of particular interest to note that 1 fly in the packed soil and 20 flies in the unpacked soil reached the surface from the depth of 34 inches. The percentage of total emergence was considerably lower at the 34-inch depth than at other depths, while the mortality was appreciably higher. Soil tempera-

ture at this depth may have affected the percentage that emerged. The erratic nature of certain of the data is difficult to explain. With cage *B–2*, where puparia were at a depth of 14 inches, only 8 per cent of those that emerged were collected on the surface; while with cages *B–3* and *B–4*, with puparia at 20 and 26-inch depths, 63 and 34 per cent, respectively, were collected. It is possible that the cages may have been invaded by predacious insects such as certain ground beetles or ants. However, at no time was there any indication of such insects having been present.

The number of puparia unaccounted for is also difficult to explain. It is probable that the pupae died shortly after the experiment was set up, in which case the pupal shell may have deteriorated sufficiently for broken parts to pass through the regular sifting screen. Since there were considerable numbers missing in each cage, it is indicative that similar factors were operating in all cases.

Time of Day of Emergence.—Limited studies were conducted to determine the time of day at which emergence took place. This was of con-

TABLE 3

SUMMARY OF TWO-HOUR OBSERVATIONS REGARDING EMERGENCE FROM SOIL ON
AUGUST 18, 19, 20, 1929

Hour	Mean air temperature, degrees F	Mean humidity, in per cent	Number of flies emerging			Per cent of total daily emergence	
			Male	Female	Total	Mean	Cumulative
Morning							
Before 6	62	86	8	4	12	3	3
6– 8	76	66	79	91	170	39	42
8–10	83	43	85	113	198	43	85
10–12	94	38	21	22	43	10	95
Afternoon							
12–2	95	37	4	5	9	2	98
2–4	88	42	1	2	3	1	99
4–6	80	54	2	3	5	1	100

siderable importance with reference to the time of day at which collection of flies from emergence cages could be most advantageously made. Information was obtained by collecting the flies every two hours from a few representative cages. These data are presented in table 3.

It is evident that the major portion of the daily emergence occurred in the forenoon before ten o'clock. Apparently this feature of emergence is related to temperature. In this instance it is distinctly advantageous to the species to emerge before soil-surface temperatures be-

come high enough to cause mortality. On several occasions when newly emerged flies were placed on the soil surface at the peak of the day's temperature, death quickly ensued. On days of relatively cool periods it was commonly observed that emergence was fairly uniform in rate throughout most of the day.

Discussion of Emergence Data.—A partial summary of the emergence data for the five-year period is presented in table 4. It is particularly interesting to note that biennial-generation individuals represent a relatively high percentage of total population and furthermore that a portion does not emerge until the third and fourth years after pupation. There were no second-generation individuals in either 1928 or 1930, when these experiments were conducted.

These studies show important relations of temperature to emergence. The data already presented suggest that the time of initial emergence is dependent somewhat upon accumulated temperature effects during dormancy. The winter seasons of 1927–28, 1928–29, and 1931–32, are classed as cold, that is, with nearly normal or subnormally low temperatures, while those of 1929–30 and 1930–31 are classed as mild, that is, with abnormally high temperatures. The medians of emergence in the former instances were reached on August 17, 24, and 16, respectively; while in the latter instances the medians were comparatively early, occurring on July 29 and 18, respectively. In this connection it is of interest to note that the percentage of annual-generation flies after so-called cold winters was approximately 60 per cent, in contrast to approximately 86 per cent after mild winters. Furthermore, after the cold winters females were more abundant than males during the early portion of the emergence season, while males predominated during the latter portion. Also the average sex ratio for these three years shows slightly more females, while the situation is reversed in 1930 and 1931. After mild winters the two sexes emerged at about the same rate.

The rate of emergence is apparently related to current soil temperature, which is directly related to air temperature. Soil temperature fluctuations usually lag 2 or 3 days after fluctuations of air temperature. After emergence has been initiated the rate is low when the mean daily air temperature is 65° F or below; however, at no time during the emergence period was the mean below 60° F except late in the season when most of the flies had emerged. At 70° F the rate increases and is rapidly accelerated by higher temperatures.

The data comparing emergence of annual and biennial-generation flies are summarized in table 5. Biennial-generation flies reached the median of emergence 7 days earlier on the average than did the annual-generation ones. Accumulated temperature effects during dormancy

TABLE 4

EMERGENCE OF WALNUT HUSK FLY, 1928–1932, SHOWING SEX RATIO AND DISTRIBUTION OF GENERATIONS

Year	Date when seasonal median reached	Total number of cages	Number of flies			Ratio ♀ : ♂	Number of healthy pupae at end of season	Approximate percentage emerged after pupae remained in soil for period of:*			
			♀	♂	Total			1 year	2 years	3 years	4 years
1928	August 17	4	759	700	1,459	52 : 48	640	69.9	28.7 –	1.2	0.2
1929	August 24	20	3,956	3,442	7,398	53 : 47	9,203	44.6	55.1 –	0.3	†
1930	July 29	15	4,318	4,647	8,965	48 : 52	1,014	89.8	9.3 –	0.9	†
1931	July 18	12	5,564	6,292	11,856	47 : 53	2,395	83.2	16.8 –	†	†
1932	August 16	15	3,688	3,712	7,400	50 : 50	3,718	66.5	33.5 –	†	
Total		66	18,285	18,793	37,078	50 : 50‡	Mean	70.8	28.7 –	0.8	0.2

* Pupal mortality was not considered in calculating these data.
† Data not available.
‡ Unweighted average.

may be offered in partial explanation of the facts; however, it is probable that genetical factors exert greater influence with respect to biennial-generation individuals than does temperature.

TABLE 5

COMPARISON OF EMERGENCE OF ANNUAL AND BIENNIAL GENERATION FROM THE SOIL FOR A FOUR-YEAR PERIOD

Year	Approximate date when 50 per cent of total emergence occurred		Difference (− = earlier, + = later) for biennial generation
	Annual generation	Biennial generation	
			days
1929	August 24	August 17	− 7
1930	July 29	July 31	+ 2
1931	July 26	July 13	−13
1932	August 12	August 1	−11
Mean			− 7

The data comparing emergence from cages distributed with respect to tree exposure are summarized in table 6. The median of emergence was reached approximately 7 days earlier in cages on the south side of trees, and 10 days earlier in those on the west side, than in those on the

TABLE 6

COMPARISON OF EMERGENCE FROM SOIL IN CAGES LOCATED ON VARIOUS EXPOSURES OF TREES

Year	Approximate date when 50 per cent of total emergence occurred			Difference (+ = later)		
	North	South	West	North as compared with south	North as compared with west	South as compared with west
				days	*days*	*days*
1931	July 26	July 19	July 16	+7	+10	+3
1932	August 21	August 14		+7		
Mean				+7	+10	+3

north side. These differences are apparently related to soil temperature since the south and west exposures receive more sunlight than the north, which is shaded by the tree a great deal of the time.

Regarding the effect of depth of burial upon emergence, the data show that the median of emergence was reached approximately 10 days earlier in the 3-inch depth cage than in the 12-inch depth one (table 7). Both cages were located identically, therefore soil temperature is suggested in explanation of the facts.

The data presented in table 7 indicate further interesting relations of temperature to emergence. It is apparent that time and rate of emergence, and also the actual number of flies that emerge from any given location in one season, are related to temperature. In 1931, which was a mild winter, there was no appreciable difference in the percentage that emerged from the north and the south locations. However, in 1932 (cold winter) 27 per cent more flies emerged from the south locations than from the north locations. Temperature during dormancy is also correlated with the percentage of individuals that constitute the annual and biennial generations respectively.

TABLE 7

Effect of Location in Soil and Age upon Percentage of Total Pupae from Which Adults Emerge

Year	Location of cage under tree		Age of pupae		Depth pupae buried in soil	
	North	South	1 year	2 years	3 inches	12 inches
	Per cent of total pupae in soil from which flies emerged					
1931	82	81	81	95	....	....
1932	52	79	65	83	83	60
Mean	67	80	73	89	83	60

Chemotropism of the Adult.—A considerable amount of research has been done in the field of chemotropic responses of the Trypetidae, particularly with regard to *Ceratitis capitata* Wied. (Mediterranean fruit fly), *Pterandrus rosae* (Ksh.) (Natal fruit fly), and others. However, definite information regarding the chemotropic reactions of members of the genus *Rhagoletis* is limited. In view of the great interest in this field and the possibility that information obtained might have a bearing on the control of *Rhagoletis completa*, this phase of the study received a considerable amount of attention. Olfactometer experiments employing either McIndoo's[28] or Ripley and Hepburn's[32] types of apparatus, or both, would have been highly desirable, but such time-consuming studies were not possible. The methods employed therefore were largely random testing of readily available volatile organic materials to determine whether or not there was an appreciable positive response by the flies. Rather simple and crude preliminary indoor laboratory tests, using the various chemicals, were carried out first.

All materials tested in the laboratory were subsequently given field trials. In some instances the less expensive materials were sprayed onto the foliage, but this method was obviously not suitable for the desired

studies. In other trials, pieces of cheesecloth of uniform size (1 foot square) were saturated with the material and hung in the trees.

For want of a better field method, all factors considered, the final tests were conducted in the following manner: A total of 500 flies approximately equal in sex ratio and of varying ages was liberated in each cage of a series of cheesecloth cages ($12 \times 12 \times 12$ feet) over bearing walnut trees. As indicated previously this type of cage apparently does
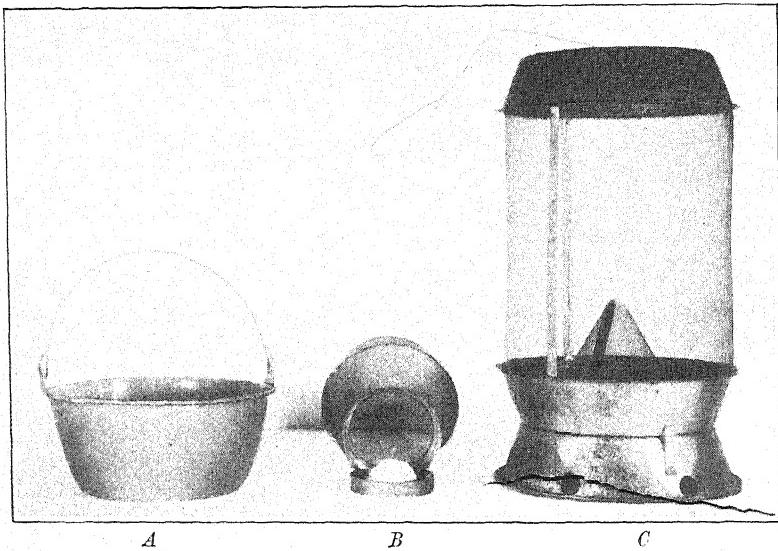
Fig. 49. Container and traps used in field chemotropic studies: *A*, open saucepan; *B* and *C*, traps. *A* and *C* are used mainly with fermenting molasses baits. *B* is used mainly in studies of various organic chemicals in large cages enclosing trees. The absorbent cotton contained in a section of petri dish, shown in the detached base, was saturated with the chemical to be tested.

not seriously affect the normal behavior of the flies. No cage was within 150 feet of another, nor in the direct path of another with reference to the prevailing winds. Two small fly traps (fig. 49 *B*) containing the chemical to be tested were hung in each caged tree, one in the lower part and the other in the upper part.

The adaptability of this type of trap was fairly well established when baited with fermenting molasses and hung in infested trees under natural conditions. A few specimens of *Rhagoletis completa* were captured, which demonstrated that *completa* was attractable and would enter traps. Fairly compact wads of absorbent cotton were saturated with the chemical to be tested and were placed in a section of a 1½ inch petri dish, which prevented direct contact between the chemical and the trap.

With the exception of a few of the very highly volatile materials, such as methyl acetate and petroleum ether, the cotton was at least moist and the odor still present at the end of 24 hours' exposure. The highly volatile materials were renewed at the end of 16 hours, before any of them was completely dissipated. Each chemical tested was exposed for 24 hours. Observations were made three times daily—in the early morning, in the midafternoon, and in the early evening before dusk— to ascertain whether or not any flies had entered the traps or were in the immediate vicinity. It is further assumed that had the material been sufficiently attractive to receive further consideration the flies would have entered the traps. Over 100 materials were tested in the above manner. All gave neutral results.

Considering the fact that the insect is practically monophagous, one would logically expect that the essential oils of the Persian walnut would produce a positive chemotropic stimulation. A small amount of walnut oil was obtained through destructive distillation of leaves and husks. Experience showed that the leaves were more readily handled and seemed to yield more oil, though neither tissue was rich in oil. The oil was tested thoroughly in the laboratory and field with neutral results.

The materials tested are as follows:

Acids
Acetic
Benzoic
Butyric
Naphthenic
Salicylic
Tannic

Aldehydes
Benzaldehyde
Butalyde
Cinnamaldehyde
Formaldehyde
Furfural
Salicylaldehyde

Alcohols
Amylic
Benzol
Butyl
Iso-butyl
Caprylic
Diacetone
Ethyl
Glycerol
Methanol
Methanol (synthetic)
Iso-propyl
n-Propyl
Tulol

Esters
Amyl acetate
Butyl acetate
n-Butyl propionate
Di-butyl phthalate
Ethyl acetate
Ethyl lactate
Ethyl sulfate
Iso-amyl benzoate
Methyl salicylate
Methyl-acetate

Hydrocarbons
Ethyl benzyl
Naphthalene
Toluene
Xylene

Substituted hydrocarbons
Bromobenzene
Chlorobenzene
Ethylene dichloride
Nitrobenzene
O-nitrochlorobenzene

Mineral compounds
Creosote
Distillate
Eocene
Gasoline

Mineral compounds (continued)

Kerosene
Petrolatum amber
Petroleum ether
Red engine oil
Tar oil

Phenols

Cresol
Phenol
Resorcinol

Amines

Ethyl benzol aniline
O-toluidine
Triethanolamine

Terpenes

Borneol
Camphor
Citronellol
Cymene
Eugenol
Geraniol
Terpineol

Essential oils

Oil aniline
Oil caraway
Oil cedarwood
Oil chamomile coctum

Essential oils (continued)

Oil citronella
Oil cloves
Oil eucalyptus
Oil fennell
Oil guajacwood
Oil lavender
Oil lemon
Oil linseed
Oil mustard
Oil origanum
Oil peppermint
Oil pine
Oil red thyme
Oil sassafras
Oil star anise
Oil sweet almond
Oil sweet orange
Turpentine
Oil thuja
Vanilla extract
Oil walnut (Eureka variety)

Miscellaneous

Ammonium hydroxide
Clensel (proprietary soap)
Distilled water
Fish oil
Pyridine
Sulfur
Quassia chips

Certain bait experiments were carried out in which ½-gallon dark-enameled, open saucepans with bails were employed (fig. 49 *A*) as traps. The following materials were tested: shredded walnut husks, shredded walnut leaves, essential oil of walnut, cane-sugar solution in concentrations of 5, 10, 25, and 50 per cent, and also the proprietary compound "Clensel," which has given such striking results in attracting large numbers of the Mediterranean fruit fly in Australia. The saucepans containing the various materials in water were hung in the lower portions of the trees. Six trees in a heavily infested walnut grove were employed in testing each material. The experiments extended over a period of twenty days at the height of fly activity, with neutral results. An occasional fly of the *completa* species was captured in the liquid in the bait pans, but this was probably an accidental occurrence since they were also found occasionally in the controls which contained water.

Preliminary tests were also conducted with fermenting baits. Frost[16] and Peterson[29] have studied this subject in great detail with reference to the moth *Laspeyresia molesta* Busck (Oriental peach moth). Frost reports in his *Laspeyresia* studies that great numbers of *Rhagoletis cingulata* Loew (light-banded cherry fruit fly) were taken in bait pans containing fermenting molasses. Ripley and Hepburn[33] in South Africa

made detailed studies of fermenting baits in relation to the trypetid *Pterandrus rosae* (Natal fruit fly). They worked primarily with treacle in combination with pollard and bran.

In these studies dealing with *Rhagoletis completa* the various materials were hung in the trees in open saucepans. The experiments were conducted in a fairly heavily infested grove at the height of fly activity and extended over a period of twenty days. Twelve trees were employed

TABLE 8

Adults Captured in Open Bait Pans Containing Fermenting Substances

Experi-ment No.	Materials	Concentrations	*Rhagoletis completa**			
			♂	♀	Total	Average per pan†
1	New Orleans molasses............... Water...	50 cc 950 cc	37	25	62	5.2
1a	New Orleans molasses............... Water... Sodium arsenite...........................	50 cc 950 cc 1 cc	24	15	39	3.3
2	Wheat bran.................................... New Orleans molasses............... Water...	90 grams 20 cc 1 liter	32	19	51	4.3
3	Middlings (Pollard)..................... New Orleans molasses............... Water...	90 grams 22 cc 1 liter	56	28	84	7.0
4	Control (water).............................	....	5	2	7	0.6

* Among other species of insects captured in these bait pans were noctuids, coccinellids, chrysopids, drosophilids, sapromyzids, ortalids, and other miscellaneous species.

† 12 pans in each experiment.

in testing each particular material or combination of materials. One container was hung in each tree. The materials used, concentrations, and results, are summarized in table 8.

The materials and concentrations used in experiments 1 and 1a of table 8 were also employed in screen fly traps (fig. 49 *C*) hung in the trees. The results obtained were essentially in accordance with those reported for the respective materials in table 8.

The data of table 8 indicate that *Rhagoletis completa* is positively chemotropic to volatile products of fermentation. The attractiveness is apparently selective with regard to sex since more males were consistently captured than females. However, the differences may not be significant as the fly population in this particular grove at that time may have consisted of an excess of males over females. The addition of sodium arsenite to the molasses-water bait appreciably reduced the rate of

fermentation, which fact in turn apparently reduced the attractiveness of the material. Other workers have reported similar results in tests with other insects.

It is recognized that the studies on chemotropic responses herein reported were not sufficient to base important conclusions upon. However, they indicate that this line of attack does not merit extensive investigation in connection with control.

. *Phototropism of the Adult.*—Limited experiments were conducted relating to the phototropic responses of the flies. Laboratory tests, in which regular Mazda electric light bulbs of clear, yellow, red, green, and blue were employed, resulted in inconclusive information. In the field tests, lights of these colors were suspended in wire-screen cages enclosing walnut trees, and hundreds of flies were liberated in the cages. Neutral results were obtained.

Small-scale field tests were conducted in an effort to determine whether or not flies would exhibit a positive response to white materials on walnut foliage. The following white materials were employed : talc, hydrated lime, arsenate of lead, and barium fluosilicate. Each of these white materials was used singly and in contrast with the black material, lampblack (carbon). Lampblack was also used singly. The end of one branch in a favorable location on each of four walnut trees in a moderately infested grove was treated until no more material would adhere to the foliage. Where the black and white materials were contrasted, a similar area in close proximity on the same tree was treated likewise with the contrasting material. These treated areas were closely observed twice daily for a period of one week in order to compare the numbers of flies present on each. The results are considered to be neutral, since the flies exhibited no particular response by frequenting treated foliage; neither were they repelled.

Anemotropism and Thermotropism of the Adult.—From laboratory anemotropic studies it was concluded that flies respond neutrally to this stimulus, except under extreme conditions wherein they are forced to orientate themselves with head in the direction of air current for protection. Simple thermotropic tests within the temperature range of 70° to 130° F showed that the flies were neither attracted nor repelled by temperatures that were not detrimental to them. However, when the temperature of their environment was above 120° F, they became excitedly active. At higher temperatures they succumbed with relatively short exposures.

Geotropism and Thigmotropism of the Adult.—On emerging from puparia in the soil, the adults indicate strong negative geotropism. The

path made by newly emerged adults has been observed in detail. Puparia placed against the surface of glass containers, and covered with very finely divided pure sand to a depth of 6 to 8 inches, afford a good method of studying the path made by the emerging adult, provided the sand is thoroughly wetted to a depth below the location of the puparia. In every instance in the many cases observed the path has always been upward though not necessarily vertical. Under field conditions the emerging adults no doubt follow the path of least resistance, taking advantage of horizontal cracks that eventually lead to cracks extending upward. The indications are that the flies do not respond to geotropic stimuli after they have reached the surface and their bodies become hardened.

A positive thigmotropism may be indicated by the behavior of the adults immediately upon emerging from the puparium. Dead bodies of newly emerged flies were often found in the center of tightly rolled cotton plugs in glass test tubes where puparia were contained without soil. Some of the test tubes were upright and some horizontal in position and each contained only small numbers of puparia in the bottoms of the tubes. Therefore space was not particularly limited. In those instances where the test tubes were upright these observations may simply indicate a negative geotropic response; however, where the tubes were horizontal positive thigmotropism is suggested.

Feeding Habits.—The food of adults under natural conditions is not definitely known. Illingworth[23] in his studies on *Rhagoletis pomonella* (Walsh) on apples states, "The surface gum [on the fruit] is apparently the only food taken when the flies are in the orchard, for when they go to the leaves it appears to be for rest and for shelter from the weather." It seems improbable that gum or wax alone would supply sufficient nutritive substances, if any at all.

Honeydew resulting from aphid infestation undoubtedly constitutes a portion of the food of *R. completa*. The walnut aphid, *Chromaphis juglandicola* (Kalt.) is always present in greater or lesser numbers throughout the period that the tree is in foliage. Sufficient numbers are usually present in the early portion of the season to excrete enough honeydew for visible amounts of sooty mold fungi to develop. Consequently quantities of honeydew and spores of fungi are available for food throughout the period of fly activity.

Yeasts occurring naturally on the foliage and bark are probably ingested and serve a nutritive purpose. Evidence of ingestion is forthcoming from Berlese's work (Caldis[9]). In his studies of souring of figs, he found that yeasts multiplied and also hibernated in the intestines of a number of species of flies. Furthermore the surface of the walnut bears

small glandular hairs. It is not improbable that the cells of these glands are readily ruptured by the fly. The contents of such cells should be rich in nutritive substances. Plant sap oozing at pruning scars and also from lesions due to invading bacterial and fungal organisms is also consumed by the flies.

Feeding may take place at any time of day; however, the flies generally feed more actively in the early morning when climatic conditions are favorable. Atmospheric moisture in the form of dew is usually present until about eight o'clock on a typical morning with sunshine. They move about very little if at all at temperatures of 50° F and below and are quite sluggish even at 55° F. However, when temperature reaches approximately 60° F after daylight they begin to move about, apparently in search of food. This movement consists of walking about accompanied by short flights. The rate at which they walk about increases as the temperature rises and apparently is also stimulated by the rapid evaporation of dew. It thus seems that the presence of moisture materially aids them in the ingestion of food; however, it may mechanically hinder locomotion. Extensive field observations indicate that feeding takes place at a time of day when flies are likely to obtain food with the least effort.

In feeding, the flies walk about on the leaves and nuts with proboscis extended downward, sampling the surface for the presence of food. When a favorable spot is located, a rapidly pulsating action takes place at the base of the mouth parts, which probably indicates that some material is being sucked up. The proboscis is frequently cleaned with the aid of the forelegs. They consume quantities of liquid by sucking up droplets of dew. In many instances also flies have been observed to emit a small amount of fluid onto the surface, then move the fleshy labella about in a rasping manner, and later suck it up again. This no doubt serves to change the desired food material into a liquid state, or causes small solids to become suspended in the liquid for consumption. This is the usual procedure when feeding upon sweetened materials that have been applied on the foliage. When the food supply is plentiful the flies engorge until their abdomens are greatly distended and a droplet of liquid usually surrounds the labella of the proboscis. Such engorged condition appears to inhibit activity temporarily to a considerable extent.

In relation to the control of the fly, it was desirable to study the action of the mouth parts with reference to consumption of more or less insoluble particles of insecticides. These ingestion studies are reported elsewhere in this paper (p. 510) since they pertain more specifically to toxicological data.

Flight and Dispersion.—On casually examining flies morphologically the impression is gained that they are well adapted for strong flight. The flights are usually of short duration. The flies are very rapid in "taking off" and also while on the wing; many of their flights are begun by characteristic "darts." They have a fast "landing speed" and come to rest on the leaf with a characteristic noise or thud. In field studies it commonly happened that flies were located very readily by the sound made on alighting on a nearby leaf.

With reference to the general migration of flies from tree to tree within an infested grove, little is definitely known. Since a satisfactory attrahent with which traps may be baited has not been found, it is difficult to obtain detailed information regarding dispersion. Flies have been observed to travel on the wing from one tree to another. Detailed counts of the degree of infestation on all trees within a grove show that the infestation within a unit area is not homogeneous. This fact may be explained by a tendency for flies to localize on the tree that they first reached on emerging from the soil. Another possible explanation would be that, because of the hardness of walnut husks of certain individual trees, the females were unable to oviposit and began to migrate in search of susceptible walnuts. The trees bearing nuts with susceptible husks were therefore located by the flies as a result of "trial and error."

In migration studies in 1929, two separated rows in a 10-acre (660 × 660 feet) block of Elberta peaches were sprayed with sugar solution. Flies were observed on all sprayed trees. This peach planting adjoined a 10-acre block of Eureka walnut trees that were heavily infested in 1928. Since the peaches were harvested before complete maturity was reached, very few of the flies present could have developed on this host. Therefore most of the adults in this peach planting either migrated there voluntarily or were subjected to a dispersing effect by the prevailing southwest winds.

An effort was made in 1930 to study the movement of flies within a small, infested Eureka walnut grove. For this purpose flies were permanently marked. Several methods were tested, namely, clipping a portion of the wing, marking the wing with India ink, amputating one leg, and other methods, none of which was satisfactory for field studies. The marking of the scutellum with India ink, however, proved satisfactory, since it did not injure the fly and was easy to detect. The scutellum is normally yellowish white and is very conspicuous, because of the contrast afforded by the darker colored body. Of the various colors tested either brick red or light green was satisfactory, though brick red was slightly more conspicuous and was used for this reason. To accomplish the marking, the flies were placed in short, wide-mouthed fruit jars, 25

individuals per jar, and these were then kept at a temperature of approximately 37° F for one-half hour. This treatment inactivated the flies, and under these conditions a small drop of the India ink was placed on the scutellum with a camel's hair brush. Four hundred flies were marked in this manner in approximately one hour. Of these, 350 flies were liberated on one medium-sized walnut tree. The remaining 50 flies were placed in the inverted battery-jar cage on walnuts in the field laboratory to study the effect of the treatment on the flies. They remained alive and behaved normally throughout the remainder of the season. The scutellum marking was permanent and was very conspicuous.

Sugar solution was sprayed onto the tips of branches in the lower, middle, and upper portions of each of the four sides of the tree on which the flies were liberated. The same solution was also placed on the lower portion on four sides of all peach and walnut trees within a radius of 100 feet. Daily observations were made in order to note the number of marked flies feeding on the artificial food in the various locations of the tree on which they were liberated; and also to note whether or not any had migrated to surrounding trees. The greatest number of marked flies observed in any one day was 12. These were feeding on the sugar in the top of the tree on the southeast portion during late afternoon. Occasionally a single marked fly was noted feeding in other portions of this tree. In no instance was a marked fly recorded from any of the surrounding trees. For studies of this nature a large number of marked flies, perhaps 8,000 to 10,000, would be necessary to yield sufficient data to be representative of normal dispersion.

In 1932, Phipps and Dirks,[30] studying dispersion of *Rhagoletis pomonella,* reported important experiments in which marked flies were used. Their data show that 27 per cent of the marked individuals that were recovered were taken within a radius of 75 yards; 57 per cent between 75 and 96 yards; and 16 per cent from 98 to 156 yards.

Yearly increase of infested acreage, as an index to normal dispersion, supplies suggestive data. However, an extensive control campaign has been conducted since 1928, which may have affected normal dispersion somewhat. Yearly surveys of the same area showed increases in infestation amounting to from $\frac{1}{4}$ to 3 miles, in a straight line, per year.

Population Studies.—For purposes of control, information regarding the relative numbers of flies present on trees in various areas was highly important. In 1928 it was observed that sweetened materials applied on the foliage served to congregate the flies. Cane sugar was most commonly used in preliminary studies. Since this substance possessed no chemotropic attraction, the fact that the flies became congregated in such

small areas as on a few leaves on the tip of a branch indicates that they moved about generally over the trees.

For these studies a grove of medium-sized trees was chosen, in which a fairly high fly population was known to exist since many walnuts showed evidence of recent infestation. On each of two adjoining trees a 20 per cent cane sugar solution was sprayed onto leaves at the tip of a branch on the north, east, south, and west locations. On three other trees in an adjacent row the material was applied only on the east and west locations. Detailed observations and counts of flies present were made at hourly intervals from 6:00 a.m. until 6:00 p.m. on two consecutive days. Early in the morning of the third day, canvas was placed under each tree and the trees were heavily dusted with high-concentration nicotine dust. Application was made under favorable conditions and before the flies had begun to move about. The indications were that practically all flies were brought down onto the canvas. The flies were collected before they revived. On the following morning the trees were again dusted and a few flies were brought down. Five days later sugar solution was again sprayed onto the same areas on the same trees and also in similar locations on other trees within the grove. Very few flies were noted on the former trees, while flies were fairly abundant on the latter. These facts indicate that the flies do not move about much from one tree to another, provided food and host conditions favoring oviposition prevail. This assumption is made in interpreting the data regarding this phase of the study.

The data obtained from these studies are presented in table 9. Considerable variation in the behavior of the flies is evident. On September 5, over twice as many flies were observed on the north and east locations as on the south and west sides of the trees. Furthermore, when only the east and west locations were observed, many more flies congregated on the easterly side. It is of interest to note that on this morning atmospheric moisture did not condense in sufficient amounts to form dew, and the sun shone brightly all day.

On September 6, a different picture is presented. A high fog prevailed throughout most of the forenoon and during the remainder of the day the sky was overcast; the sun did not shine brightly at any time. This condition apparently affected the normal habits of the flies, particularly with respect to their movement about the trees. Less than one-half as many flies were observed as on the preceding day, and there were no significant differences in the numbers frequenting any of the respective locations except in the southerly location.

On both days appreciably more flies were observed in the afternoon than in the forenoon. A summary of the composite data secured is

TABLE 9

RESULTS OF OBSERVATIONS ON TWO CONSECUTIVE DAYS CONCERNING THE ACTIVITY
AND DISTRIBUTION OF ADULTS ON WALNUT TREES (1930)

Hour	Temperature, degrees F	Relative humidity, in per cent	Two trees with total population of 146 flies					Three trees with total population of 230 flies		
			North	East	South	West	Total	East	West	Total
			Flies observed, in per cent of daily total*							

September 5

6 a.m.	56	56	0	0	0	0	0	1	0	1
7 a.m.	63	44	1	0	3	1	5	0	0	0
8 a.m.	75	41	1	3	1	1	5	0	0	0
9 a.m.	84	35	4	1	1	1	8	2	1	3
10 a.m.	90	35	6	7	1	5	19	5	6	11
11 a.m.	92	35	4	7	1	7	19	6	4	10
12 a.m.	94	33	9	9	1	2	21	7	3	10
Total a.m.*	*M* 79	*M* 40	25	27	9	16	77	19	14	35
1 p.m.	95	35	5	8	3	5	22	6	3	10
2 p.m.	93	36	9	14	1	4	30	8	6	14
3 p.m.	92	40	6	10	3	3	21	9	3	12
4 p.m.	91	42	5	10	3	5	23	7	4	11
5 p.m.	85	47	7	8	3	4	23	6	3	9
6 p.m.	77	58	7	3	1	1	13	5	4	9
Total p.m.*	*M* 89	*M* 43	38	54	14	23	130	41	23	64
Daily total*	*M* 84	*M* 41	64	81	23	39	208	62	37	99

September 6

6 a.m.	55	63	0	0	0	0	0	0	0	0
7 a.m.	60	58	0	0	0	0	0	0	0	0
8 a.m.	69	51	0	2	0	0	2	1	0	1
9 a.m.	75	42	1	2	3	0	5	1	1	2
10 a.m.	83	47	5	3	2	0	11	1	1	3
11 a.m.	86	46	3	3	1	3	10	2	1	3
12 a.m.	87	49	3	1	0	2	7	2	3	5
Total a.m.*	*M* 74	*M* 51	12	12	7	5	36	7	6	13
1 p.m.	85	47	1	3	1	6	12	2	1	3
2 p.m.	83	49	2	3	0	4	9	2	2	4
3 p.m.	82	55	2	5	2	8	16	2	1	3
4 p.m.	78	73	3	3	1	4	12	1	1	2
5 p.m.	71	78	3	1	1	1	5	1	3	3
6 p.m.	65	82	2	0	1	1	3	0	1	0
Total p.m.*	*M* 77	*M* 64	14	16	5	23	58	7	9	17
Daily total*	*M* 75	*M* 57	26	27	12	28	94	14	15	30

* Totals include those flies that may have been observed more than once during the interim, consequently exceeds 100 per cent in some instances.

graphically presented in figure 50. The number of flies observed on both days increased with rising temperature, reaching the daily peak at 2 p.m. and 3 p.m., respectively, and decreased with lowering temperature. The inverse relation generally existed with regard to relative humidity. The data indicate that absence of dew in early morning, followed by favorable temperature and sunshine, stimulated movement
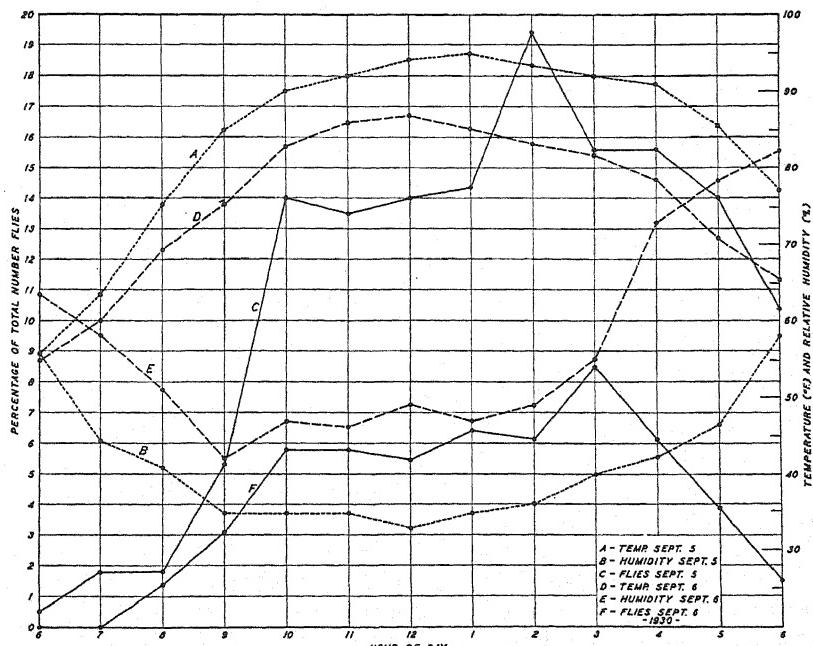
Fig. 50. Comparison of relative numbers of flies observed on the same walnut trees on two consecutive days, together with prevailing temperature and humidity (1930).

incident to acquiring food throughout most of the day. When dew was present and the temperature was favorable, movement was stimulated somewhat, though lack of sunshine apparently was related to the extent or degree of movement. However, it may be argued that sunshine had no connection with the matter and that lack of detectable movement may have been due to the fact that the flies had acquired the desired food and consequently had no need of moving about extensively.

For comparing relative populations in experimental control plots in 1929 and 1930, sugar solution was applied on a small area of foliage on the east side of representative trees. The numbers of flies observed served as an index to the population in any given plot. These studies were made during August, and on days when an appreciable quantity of dew was

present at sunrise. The temperature rose rapidly and as a result the dew evaporated quickly. Under these conditions greater numbers of flies could be observed over a period of several hours in early morning than at any other time of the day. This method was used extensively in 1929 and 1930. Since the data pertain specifically to control studies, they are treated under that phase of the subject (pp. 515–535).

Longevity of Adult.—Under field laboratory conditions, in the battery-jar type of cage, a few flies were kept alive in 1930 from July 22 to October 15, a total of 85 days. The average length of life under the most favorable laboratory conditions known was approximately 40 days. Virgin females lived on the average approximately 35 days, and the oldest one on record was 85 days old.

In cheesecloth cages ($12 \times 12 \times 12$ feet) enclosing small walnut trees, a few adults were alive at the end of 70 days, when the cages were removed.

The length of life under natural field conditions is not known. However, it seems reasonable to believe that an average span of life of from 30 to 40 days would be approximately normal. This is based on the assumption that the foregoing artificial conditions pertaining to longevity are probably as favorable on the whole as field conditions, or slightly more so.

Effect of Food on Longevity.—A simple experiment was conducted to determine the length of life of flies without food or water. One hundred flies of approximately equal sex ratio were collected immediately upon emergence from the soil. Equal numbers of these were placed in each of two inverted battery-jar cages. Approximately 50 per cent of the flies were dead at the end of 48 hours, while over 90 per cent were dead at the end of 52 hours. A few flies lived nearly four days without food.

In earlier studies granulated cane sugar, and later cube cane sugar, was put into one section of a small petri dish in battery-jar cages for food. Absorbent cotton saturated with water was put into another section of petri dish to supply moisture. Granulated sugar deliquesced and became sticky much more rapidly than cube sugar. Any sticky substance within the cage was undesirable, since the flies became trapped or, in walking about, spread the material throughout the cage to such an extent that they were frequently incapacitated when their wings came into contact with it. Therefore sticky food substances constituted a hazard which was eliminated by more frequent feeding, following the method to be described in the nutritional studies. In these studies flies lived longest under battery-jar-cage conditions in an outdoor shaded laboratory when a solution of 10 per cent technical sucrose was supplied as food. However, only a few eggs were produced on this diet. Moisture

is very essential in the economy of the fly, both for food and for maintenance of optimum humidity conditions; which role is of most importance is not entirely clear.

Effect of Temperature on Longevity.—The temperatures that normally prevail during the period of fly activity rarely drop lower than 40° F, and are not low enough to exert a deleterious effect on longevity. In fact, general observations over a four-year period in the laboratory and field indicate that length of life during the later (cooler) portion of the season appreciably exceeds that of the fore and middle portions. Under laboratory conditions, daily maximum temperatures around 95° to 100° F shortened life materially; and at 105° F for several hours, death ensued unless a high relative humidity was maintained. The highest temperature recorded during these studies was 114° F for nearly two hours' duration. The flies died in all of the cages except those where abundant moisture prevailed; in the latter the flies remained inactive while clustered on the saturated wad of absorbent cotton in the cage. In cheesecloth emergence cages the newly emerged flies generally left the walls of the cage and rested on shaded soil when the air temperature exceeded 100° F for half an hour or longer. At slightly higher temperatures, in emergence cages, many of the flies became inactivated and later died; however, a few revived before nightfall.

Effect of Relative Humidity on Longevity.—Repeated experiments under identical conditions early in this study demonstrated the superiority of the inverted battery-jar cage over a screen cage of similar size and shape. The nature of this difference appeared to be related to high relative humidity. Accordingly a comparison was made of temperature and humidity conditions existing during a period of six days in these two types of cages, together with those existing within the surrounding walnut grove. These records are presented in table 10.

It is evident from these data that the battery-jar cage maintained a higher relative humidity with only slight fluctuations. Also the temperature was somewhat reduced. The differences shown for the screen cage and walnut-grove conditions are slight and are no doubt the result of location, since the field laboratory was situated in the shade of a large walnut tree.

Further evidence on the role of humidity in the economy of the fly is forthcoming from other sources. As previously reported, flies lived for relatively long periods of time in cages (12 × 12 × 12 feet) over small walnut trees when the cages were covered with cheesecloth. However, under identical conditions except that the cages were covered with 16-mesh wire screen, most of the flies were dead in 2 or 3 weeks' time. The cheesecloth covering apparently served to maintain a favorably high

relative humidity in the immediate environs of the tree. Field observations indicate that small walnut trees do not afford environmental conditions conducive to longevity. A suggested explanation is that the rela-

TABLE 10
COMPARISON OF TEMPERATURE AND HUMIDITY IN BATTERY-JAR CAGES AND IN WIRE-SCREEN CAGES, WITH NORMAL WALNUT-GROVE CONDITIONS; 1931

Day	Hour	Temperature			Relative humidity		
		Battery-jar cage*	Screen cage*	Walnut grove†	Battery-jar cage*	Screen cage*	Walnut grove†
		degrees F	*degrees F*	*degrees F*	*per cent*	*per cent*	*per cent*
July 25	2 p.m.	102	104	103	93	38	39
	5 p.m.	90	90	88	97	50	51
July 26	9 a.m.	82	85	89	98	74	58
	1 p.m.	95	97	101	92	45	47
	5 p.m.	94	93	93	98	56	55
July 27	9 a.m.	77	79	82	98	81	71
	1 p.m.	96	97	98	92	51	52
	5 p.m.	84	84	84	94	51	53
July 28	8 a.m.	78	78	76	98	74	65
	12 m.	90	90	89	94	53	49
	5 p.m.	86	85	85	94	51	52
July 29	8 a.m.	75	76	72	98	95	71
	12 m.	89	90	86	95	54	49
	6 p.m.	82	81	77	98	75	69
July 30	8 a.m.	72	73	69	98	95	77
	12 m.	86	88	83	94	59	53
	4 p.m.	85	84	82	92	64	58
Mean‡	All readings	88	89	88	95	59	54

Differences between readings

Readings compared	Temperature	Relative humidity
	degrees F	*per cent*
Battery-jar cage compared with screen cage	−1	+36
Battery-jar cage compared with walnut grove	0	+41
Screen cage compared with walnut grove	+1	+ 5

* The battery-jar and screen cages were in a screened, shaded laboratory. Lambrecht Polymeters (mercury thermometer and hair hygrometer) were used.
† Records from hygrothermograph in standard shelter in walnut grove.
‡ Mean includes many readings during this period that were omitted from table for brevity.

tive humidity within the inner foliage is appreciably lower during the warmer portions of the day than in the case of a medium or large-sized tree under identical atmospheric conditions. The relative amounts of moisture released in transpiration from small trees and large trees may effect such differences in this semiarid region.

Suggestive data regarding humidity were obtained in 1931. Dual atmometer bulbs were stationed in each of the following locations: in total sunlight, 5 feet above soil surface; in center of medium-sized walnut tree, 13 feet above soil surface; and in upper portion of the same tree, 20 feet above soil surface. The evaporation of water from the surface of these bulbs throughout the major portion of the season of fly activity was obtained. These data are presented in table 11.

TABLE 11

EVAPORATION OF WATER FROM ATMOMETER BULBS LOCATED IN DEEP SHADE, PARTIAL SHADE, AND TOTAL SUNLIGHT, IN WALNUT TREE

Bulb* No.	Total sunlight, 5 feet above soil	Deep shade, center of walnut tree, 13 feet above soil	Partial shade, upper portion of walnut tree 20 feet above soil
	Actual loss of water per bulb from August 1 to September 17 (48 days), 1931		
	cc	*cc*	*cc*
142	1,616	....	....
162	1,516	....	....
157	....	1,240	....
158	....	1,016	....
136	....	....	1,771
Mean loss, entire period	1,566	1,128	1,771
Mean loss per day	33	24	37

* All bulbs were of S 30 series, with 0.80 correction factor.

There was an appreciable increase in the rate of evaporation of water from the atmometer bulbs located in total sunlight and in the upper portion of the tree in contrast to those located in the center of the tree. The relative humidity probably was materially higher in the inner foliage of the center of the tree than elsewhere.

Nutritional Studies.—In the earlier portion of the biological studies ordinary cane sugar was used as food for the flies. Other materials, such as glucose, honey, and raisins were used in preliminary studies but none seemed quite equal to sucrose in general adaptability for artificial food. However, sucrose was not entirely satisfactory, particularly with respect to fecundity of females. Fluke and Allen[15] in 1931 reported encouraging results with yeast suspended in honey water as artificial food for *Rhagoletis pomonella*.

The available information regarding insect nutrition (mainly based upon Uvarov[39]) indicates that nitrogen in the diet of adults is unnecessary for health and longevity and in fact generally shortens the life of the individual somewhat in comparison with an exclusive carbohydrate

food. An excess of proteins may be deleterious to longevity. However, nitrogen is essential for reproduction since it stimulates egg development.

Carbohydrates, aside from being necessary for longevity, have been shown to be essential for the development of genital products. The limited data indicate that sucrose and levulose are more valuable in the diet than other sugars, while glycogen and starch are of negative value without the presence of the corresponding enzymes. The role of fats is scarcely understood at all.

Little is known of the role of minerals; however, the following have been shown to affect reproduction favorably when consumed in either the larval or adult stages: potassium, phosphoric acid, magnesium, ferrous chloride, ferrous sulfate, copper sulfate, sodium hydroxide, and potash. Of the known vitamins essential in mammalian nutrition positive evidence is available regarding only one, vitamin B, as of value in insect nutrition. Yeast supplies vitamin B. However, symbiotic microörganisms may supply the necessary vitamins, in which connection practically no information is available.

In an effort to obtain data regarding the nutrition of *Rhagoletis completa*, preliminary studies were outlined and conducted in 1931. Limited time prevented a continuance of the work in 1932. There were 85 individual tests involving a total of 3,181 flies or an average of 37 flies per test. All tests were conducted in an outdoor screened laboratory located in a walnut grove in the infested area. The flies were placed in the test cages on the day that they emerged from the soil and before they took any food except negligible amounts that may have been present on the walls of the cheesecloth emergence cage. The standard method of supplying food was to saturate a wad of sterile absorbent cotton with a measured amount of the nutrient media and place it in the cage in one half of a petri dish (fig. 32, p. 402). Every second day the media was replaced with a fresh supply. Tap water was used with the technical grades of materials, while distilled water was used with chemically pure ones. All cages were thoroughly washed in tap water at weekly intervals and dried in the sun. Clusters of walnuts in susceptible condition for oviposition were thoroughly washed before being placed in the cages. These were changed at weekly intervals. Nuts removed from the cages were kept for several weeks for further studies on larval development.

It is realized that the condition of these tests, particularly with reference to control of environmental factors, was not satisfactory for precise nutritional experiments. Furthermore it was not possible to maintain an oviposition record for individual females, owing to the relatively

large number of tests involving many flies. Therefore, for comparative purposes the average number of eggs per female was calculated by dividing the total number of eggs deposited in a single test by the number of females alive when oviposition began. Thus the calculated number of eggs per female was considerably lower than the actual number. However, the data obtained should at least be indicative of the relative values of the various materials and combinations tested.

The detailed data obtained in these studies is rather voluminous and for that reason is not incorporated in this publication. Therefore, only the most important information pertaining to the experiments and a general summary of the results will be given. These data are presented in table 12.

In no instance was oviposition stimulated to the degree that is apparent under natural conditions. The maximum number of eggs recorded per female in these tests was 30. In all tests only a small number of eggs were produced per female. Further studies regarding the role of the tested materials in the nutrition and metabolism of the fly are necessary before definite conclusions are warranted. A brief summary of these studies, treated according to food groups, follows.

Results with Proteins in Foods.—When nitrogen was added to the carbohydrate (sucrose) diet in battery-jar cages in the form of yeast (experiments 1 and 3) the flies lived less than one-half as long and deposited only one-third as many eggs. When quartz-glass cages were used (experiment 4) a material increase in longevity and fecundity was evident in comparison with battery-jar cages (experiment 3) thus possibly indicating a slightly beneficial effect from ultraviolet or other light under these conditions. Yeast added to a honey diet in battery-jar cages (experiments 22 and 24) decreased longevity somewhat and fecundity to the extent of approximately one-third. Limited amounts of nitrogen are present in honey, mainly in the few pollen grains remaining. When glycocoll, a simple amino acid, was added to a C.P. sucrose diet (experiments 9 and 10) longevity was decreased considerably but the number of eggs deposited was doubled. Nitrogen supplied as urea (experiments 9 and 15) did not decrease longevity though fecundity was nearly doubled. When furnished as ammonia (experiments 9 and 16) longevity was not affected though fecundity was increased approximately three times. In these tests yeast apparently decreased longevity and fecundity; glycocoll decreased longevity but increased fecundity; while both urea and ammonia increased fecundity, but did not affect longevity.

Results with Carbohydrates in Foods.—In only a few instances (experiments 5, 8, and 14) were there appreciable differences between technical and chemically pure sucrose, under either battery-jar or

TABLE 12
SUMMARY OF DATA FROM NUTRITIONAL STUDIES

Experiment No.	Materials used	pH of media	Type of cage	Number ♀	Number ♂	Eggs per ♀	Number of days elapsed when mortality equalled approximately 50 per cent	90 per cent
1	Sucrose (technical) 10 per cent.	6.3	Battery jar	78	100	3.1	24	57
2	Sucrose (technical) 10 per cent.	6.3	Quartz glass	16	19	2.1	25	54
3	Sucrose (technical) 10 per cent, yeast (dry) 3 per cent.	4.9	Battery jar	50	99	1.3	9	24
4	Sucrose (technical) 10 per cent, yeast (dry) 3 per cent.	4.9	Quartz glass	15	17	3.2	19	35
5	Sucrose (technical) dry.	6.6	Battery jar	49	71	30.0	5	9
6	Sucrose (technical) 10 per cent.	6.3	Screen cage hung in tree.	27	30	9.1	28	58
7	Sucrose (technical) dry.	6.6	Screen cage hung in tree.	86	138	0.0	5	8
8	Sucrose (technical) 10 per cent, solution B* 90 per cent.	4.6	Battery jar	49	62	14.0	25	40
9	Sucrose (C.P.) 10 per cent.	6.9	Battery jar	56	81	3.7	21	42
10	Sucrose (C.P.) 10 per cent, glycocoll 0.5 per cent.	6.1	Battery jar	50	53	7.0	7	34
11	Sucrose (C.P.) 10 per cent, glycocoll 0.5 per cent, solution A† 90 per cent.	5.2	Battery jar	32	46	14.0	18	31
12	Sucrose (C.P.) 10 per cent, glycocoll 0.5 per cent, solution B* 90 per cent.	5.1	Battery jar	48	53	5.3	22	40
13	Sucrose (C.P.) 10 per cent, solution A† 90 per cent.	4.9	Battery jar	39	42	9.3	25	44
14	Sucrose (C.P.) 10 per cent, solution B* 90 per cent.	5.0	Battery jar	35	45	4.5	24	37
15	Sucrose (C.P.) 10 per cent, urea 560 p.p.m.	5.5	Battery jar	39	51	6.8	18	42
16	Sucrose (C.P.) 10 per cent, ammonia 1,000 p.p.m.	9.5	Battery jar	44	49	11.7	20	47
17	Sucrose (C.P.) 10 per cent, levulose (C.P.) 10 per cent, dextrose (C.P.) 10 per cent, dextrin (C.P.) 10 per cent, urea 560 p.p.m.	4.7	Battery jar	38	54	11.7	23	37
18	Levulose (C.P.) 10 per cent, solution B* 90 per cent.	4.5	Battery jar	82	115	11.2	9	33
19	Dextrose (C.P.) 10 per cent, solution B* 90 per cent.	3.6	Battery jar	54	83	5.2	9	20
20	Dextrin (C.P.) 10 per cent, solution B* 90 per cent.	3.8	Battery jar	45	66	0.0	5	6
21	Levulose (C.P.) 10 per cent, dextrose (C.P.) 10 per cent, dextrin (C.P.) 10 per cent, urea 560 p.p.m.	4.4	Battery jar	47	63	0.0	3	5
22	Honey 10 per cent.	4.0	Battery jar	115	133	7.2	18	37
23	Honey 10 per cent.	4.0	Quartz glass.	15	20	3.0	18	48
24	Honey 10 per cent, yeast (dry) 3 per cent.	4.3	Battery jar	51	57	4.8	13	24
25	Honey 10 per cent.	4.0	Screen cage.	61	75	4.1	14	30
26	Honey 10 per cent.	4.0	Screen cage hung in tree.	52	55	16.6	22	52
27	Water (tap).	6.3	Battery jar	56	64	0.0	3	5
28	Solution B*.	5.2	Battery jar	50	61	0.0	3	5

* Nutrient solution B is identical to A, omitting Zn and Cu.

† Nutrient solution A consisted of the following chemicals in parts per million: NO_3, 144; Mg, 11; Cl, 2; Na, 1.4; Ca, 80; K, 93; PO_4, 53; Mn, 0.03; SO_4, 72; Fe, 3; Zn, 1; Cu, 3.

quartz-glass cage conditions. In these experiments (experiments 8 and 14) mineral nutrient solution *B* was included. Longevity was about equal in both; however, where technical sucrose (experiment 8) was used, approximately three times as many eggs were deposited. Technical sucrose may contain certain impurities that are essential for egg production.

When technical sucrose was placed in battery-jar cages in a dry condition (experiment 5) longevity was greatly reduced and fecundity was greatly increased. A total of 30 eggs per female was obtained, which was more than from any other experiment in the series. When placed in screen cages in trees in dry condition (experiment 7) practically all flies died before reaching egg-laying maturity. In this instance mortality was probably due to lack of moisture. Under identical conditions, except that a 10 per cent sucrose solution was used instead of dry sucrose (experiment 6), the longevity factor was approximately equal to that of battery-jar cages in the laboratory (experiment 1), while the egg production was increased three times.

Honey, in comparison to sucrose, in battery-jar cages (experiments 1 and 22) materially reduced longevity though it more than doubled egg production. In comparing honey with sucrose in quartz-glass cages (experiments 2 and 23) negligible differences existed in longevity, while slightly more eggs were deposited in the honey tests. Honey-yeast compared with sucrose-yeast (experiments 3 and 24) showed a negligible difference in longevity, but in the former egg production was increased nearly four times. Honey in screen cages in trees in comparison to sucrose under identical conditions (experiments 6 and 26) showed slightly reduced longevity, though the fecundity was nearly doubled.

When honey was fed in quartz-glass cages, in comparison to battery-jar cages (experiments 22 and 23), longevity was slightly increased though approximately half as many eggs were deposited.

Under screen cage conditions in the laboratory, as compared with screen cages in trees (experiments 25 and 26) with honey for food, the flies lived considerably longer and deposited four times as many eggs under the latter conditions. Screen cages in trees, both with honey and sucrose (experiments 6 and 26), as compared with battery-jar cages in the laboratory (experiments 1 and 22), appeared to produce conditions more conducive to egg production. These facts suggest a relation of sunlight or certain of its component rays to fecundity of the flies.

Several sugars were employed singly with mineral nutrient solution *B*, which included nitrogen, and also combinations of these sugars without minerals though with nitrogen as urea. Levulose plus solution *B* in comparison with sucrose plus *B* (experiments 14 and 18) reduced lon-

gevity materially, though egg production was more than doubled. Flies fed dextrose plus solution *B* lived only approximately half as long as those fed sucrose plus solution *B* (experiments 14 and 19); however, negligible differences existed in the numbers of eggs deposited. Dextrin plus solution *B* (experiment 20) was apparently of little value as food, since flies died nearly as rapidly as in the control tests of water and of solution *B* (experiments 27 and 28). In the combination of sucrose, levulose, dextrose, dextrin, and urea (experiment 17) longevity was slightly increased and fecundity was unaffected in comparison to levulose plus solution *B* (experiment 18), while in comparison with sucrose plus urea (experiment 15) the longevity factor was practically equivalent, though fecundity was nearly doubled. However, in the same combination minus sucrose (experiment 21) all flies died before reaching egg-laying maturity.

These tests indicate that sucrose, levulose, dextrose, or honey is essential for longevity and fecundity; that both honey and levulose reduced longevity slightly in comparison with sucrose, although fecundity was increased; that dextrose reduced longevity in comparison with sucrose without affecting fecundity; and that flies cannot survive on dextrin alone.

Results with Minerals in Foods.—Mineral nutrient solutions *A* and *B* are regular media employed in plant-nutrition research. The only difference between these solutions is the omission of zinc and copper in solution *B*. Experiments were planned in which each mineral would receive consideration; however it was not possible to carry these out. Sucrose plus solution *A,* when compared with sucrose plus solution *B* (experiments 13 and 14), showed a slight increase in longevity and doubling of egg production. Solution *A* added to sucrose-glycocoll (experiments 10 and 11) increased longevity slightly and doubled egg production. Solution *B* added to sucrose-glycocoll (experiments 10 and 12) slightly increased longevity and decreased fecundity. The data indicate somewhat increased longevity and fecundity as the result of zinc and copper in the diet.

Results with Foods of Varying pH.—The hydrogen-ion concentration of the various media ranged from pH 3.8 to pH 9.5. However these studies fail to show that any relation exists between longevity or fecundity and the pH of media throughout the range tested.

Male Reproductive System.—The male reproductive system is diagrammatically shown in figure 51. The internal portion of this system occupies most of the space in the posterior abdominal cavity. The testes are relatively large, yellowish, kidney-shaped organs surrounded by tracheae that appear to anastamose interiorly among the testicular folli-

cles. The testes unite directly with the seminal vesicles at their inner anterior ends, where a constriction occurs. The seminal vesicles are somewhat club-shaped enlargements of the vasa deferentia. One fairly large group of accessory glands, consisting of convoluted tubes, occurs at the
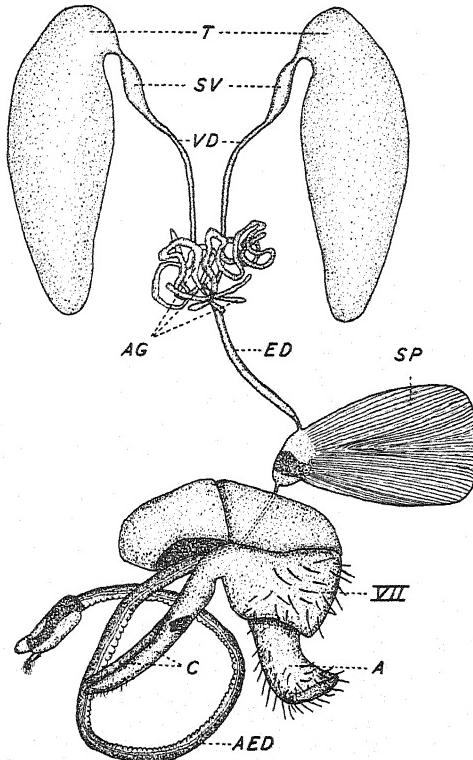
Fig. 51. Reproductive system and anus of male *Rhagoletis completa:* A, anus; AED, aedeagus; AG, accessory glands; C, claspers; ED, ejaculatory duct; SP, seminal pump; SV, seminal vesicles; T, testes; VD, vasa deferentia; VII, seventh abdominal segment.

union of the vasa deferentia and the ejaculatory duct. The seminal pump is a large, flattened, somewhat fan-shaped organ, consisting primarily of the chitinous ejaculatory apodeme, surrounded by muscular tissue. The ejaculatory duct passes through the proximal end of the seminal pump shortly before it protrudes from the body, where it becomes chitinized to form the aedeagus.

The external genital organs consist of the claspers and aedeagus. The claspers are a pair of prominent chitinous appendages on the venter of

the seventh abdominal segment. They serve to hold the ovipositor in place during copulation. The aedeagus is a long, coiled, chitinized organ
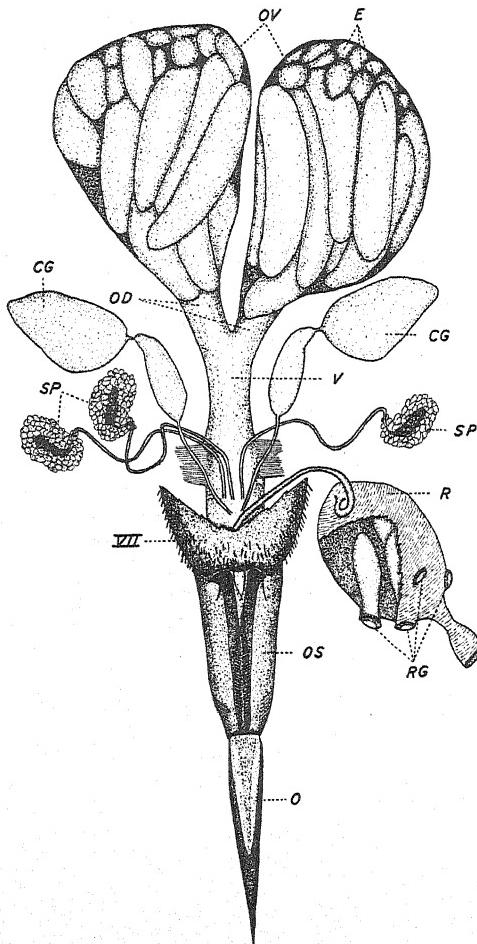
Fig. 52. Reproductive system and portion of
of hind intestine of female *Rhagoletis completa*:
CG, colleterial glands; E, ova in various stages
of development; O, ovipositor; OS, ovipositor
sheath; OD, oviducts; OV, ovaries; R, rectum;
RG, rectal glands; SP, spermathecae; V, va-
gina; VII, seventh abdominal segment. (The
arrangement of ova in the tubes is shown in
fig. 53.)

attached to a supporting framework within the sixth and seventh ab-
dominal segments. At the distal end it bears a small brush of stiff hairs.

Female Reproductive System.—The female reproductive system is
diagrammatically shown in figure 52. The internal portion of this sys-

tem consists of two pyriform ovaries connected by short oviducts to the vagina, which leads into the ovipositor. There are three spermathecae and a pair of colleterial or accessory glands which lead into the distal portion of the vagina through ducts. Each ovary consists of approximately twenty-four egg tubes which are maintained in one unit by connective tissue and many branching tracheae. Each egg tube may contain a series of eggs in various stages of development from the germarium in the very anterior end where no differentiation is evident, to a mature egg in the posterior end (fig. 53); usually four or five eggs in various
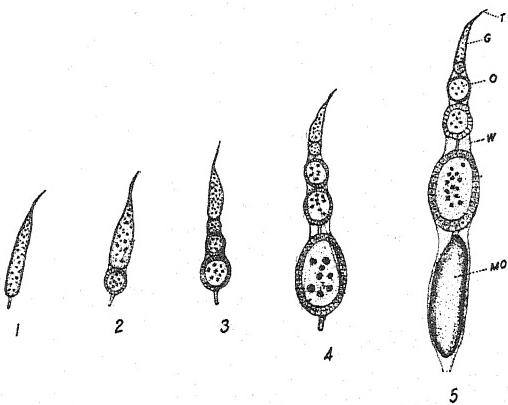
Fig. 53. Development of oöcytes in ovarian tubes with respect to number of days after female emerged from soil: 1, two days; 2, six days; 3, ten days; 4, fourteen days; 5, eighteen days. G, Germarium; MO, mature ovum; O, oöcyte; TF, terminal filament; W, wall of ovarian tube.

stages of development are present in each egg tube. When mature the eggs pass down the oviduct into the vagina, where fertilization takes place.

The external genital system is very simple, and consists of the ovipositor and sheath. The ovipositor is a slender sharp-pointed organ of chitinous structure with an opening on the ventral surface. It is attached to the last (VII) abdominal segment by the membranous sheath. This structure bears many short, triangular, chitinous projections on its surface. The ovipositor is usually telescoped into the sheath, which is in turn telescoped into the last abdominal segment.

Copulation.—Under laboratory cage conditions the time intervening normally between emergence of females and first coition with the male is from 7 to 14 days. An experiment was conducted to obtain information relative to the age of males and females at the time of copulation. Twenty-five males and a like number of females were used in each test.

In cage A newly emerged males were confined with 9-day-old virgin females. Six days later two pairs were observed copulating and fertile eggs were deposited 2 days after copulation. However, copulation was more common after 10 days had elapsed. This demonstrates that males are capable of the act at least within 6 days after emergence.

In cage B, newly emerged females were confined with 9-day-old unmated males. The following day a male finally succeeded in copulating with one of the females, after a struggle on the part of the female to prevent the act. Each succeeding day for 4 days after the experiment began, females were observed struggling unsuccessfuly to prevent copulation. However, after the females were 4 days old, they did not seem to resist so vigorously. Fertile eggs were deposited within 12 days after the experiment began.

Under laboratory conditions, copulation has been recorded at every hour of daylight at temperatures from 60° to 103° F. In the field also it may take place at almost any time of the day under varying conditions. Normally it is more commonly observed in the late afternoon, since it usually follows oviposition. In midseason in a grove with a large fly population, after about 4 p.m., many of the walnuts will have one or two males perched upon them. It is very interesting to observe how a male maneuvers in an effort to keep other males off while waiting for a female to alight on the nut. When he first takes up his position on the nut in the afternoon he is usually not very active until after one or two combats, following which he patrols the nut very effectively. He walks spryly about, with wings extended slightly upward and outward, moving them by sudden jerks, much after the fashion of a strutting peacock.

When another male alights on the nut, the original occupant extends his wings high over the body and approaches his opponent cautiously, finally darting into him bodily in an effort to dislodge him; or they approach each other and fight by standing up on their hind legs (venter to venter) and using their fore and middle legs with which to strike (fig. 54 B). In many instances they lose their balance and both fall off the nut, in which case the original occupant usually returns immediately to resume his vigil. If they do not fall off, the battle sometimes lasts several minutes, until one either is dislodged or is cowered and flies away. On several occasions as many as five males have been observed in one group, fighting one another while standing on their hind legs. Often coccinellid beetles appearing on the nut in search of aphids are attacked and driven away by the male fly.

When a female alights on the nut, the male behaves very differently. He apparently becomes very excited, as is evidenced by his actions. The object of her presence there is to feed or oviposit, and she usually takes

up her normal actions pertaining thereto without any apparent notice of the male's presence. The male approaches the female very cautiously, moving quickly sideways and apparently directing his path of approach so as to overtake the female by surprise from the rear. In many instances the female has been observed to detect the male's presence, turn and dart into him bodily, and fly away after the collision. In such cases
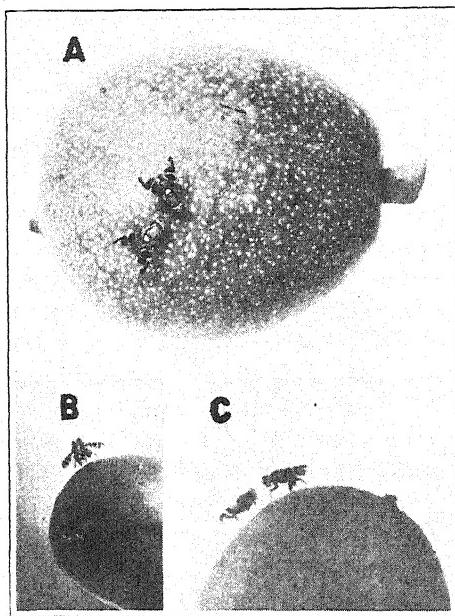
Fig. 54. Characteristic habits of adults of *Rhagoletis completa*: *A* and *C*, male and female in characteristic position on a walnut during oviposition; *B*, males fighting for supremacy of the walnut.

the female is probably either too young for copulation, is anxious to deposit a batch of eggs, or has recently been fertilized. However, the female is usually ready for copulation immediately after depositing eggs. During oviposition a male usually stands close by (fig. 54 *A–C*) or moves about, at no time getting very far away from the female. As soon as the ovipositor is withdrawn from the walnut husk, the male mounts her and generally no effort is made to resist him. She extends her wings laterally and raises the end of her abdomen, with ovipositor protruding, to aid in the union. With the hind pair of legs the male guides the tip of her abdomen so that the extended ovipositor comes to rest in the claspers of his genital organs. The intromittent organ or aedeagus is quickly

inserted and copulation ensues, usually occupying a period varying from 2 to 15 minutes. On several occasions a male has been observed to mount a female while she was in the act of oviposition and withdraw her ovipositor by force to enter into coition.

In virgin-female studies an experiment was conducted with respect to egg development in which unmated females were confined with males. Twenty-five females, 30 days old, were placed in a cage with as many males. The following afternoon, and each succeeding afternoon for several days, a few pairs were observed copulating. In each instance they remained in coition for periods of 30 to 40 minutes, which is much longer than in normal cases.

It is not definitely known how often copulation is necessary. In many observed cases, both in the field and in the laboratory, the females submitted to the act passively immediately after oviposition. This evidence, and the very promiscuous tendencies of the males indicate that it is of frequent necessity with females that are freely ovipositing, and probably takes place after depositing each batch of eggs. Under laboratory conditions in oviposition studies where one or two females were caged with males and with susceptible host material, copulation occurred very frequently, sometimes daily, over periods of time. However, only a few eggs, often none, were deposited during the lifetime of females even when they remained alive for 30 days or longer.

Preoviposition Period.—The development of eggs in the reproductive system, with reference to time elapsed after emergence, received considerable attention. This study had a very important bearing on the control of the fly. Flies of known age were dissected at two-day intervals in the early part of the study to obtain information pertaining to the rate of egg development.

The method of dissection that proved satisfactory and was used throughout was as follows: Regular embedding paraffin was melted and a layer approximately ¼ inch thick was allowed to harden in the deep-type Syracuse watch glass. The flies were killed with ether or cyanide and placed on their backs in rows on the hard paraffin. With a heated needle they were attached to the paraffin so as to cause them to remain stationary for dissection. Water was placed in the watch crystal to such a depth that the flies were completely submerged. All dissecting was performed with the aid of a wide-field binocular microscope. A dissecting needle, ground and honed to a cutting edge on the point, served to incise the abdomen and, with the aid of sharp-pointed forceps, to remove the digestive system and certain muscles and tracheae. The ovaries, which lie near the dorsal surface, are readily observed when the digestive system is removed.

For general studies a few egg tubes may be teased out with cactus-spine needles, and studied *in situ* under higher magnification of the binocular. However, for quick detailed studies, it is more satisfactory to remove an ovary and place it in some temporary mounting medium on a microscope slide. The egg tubes can be teased out of the mass under the binocular before the cover slip is placed over the preparation. The mount is then studied under the desired magnification with the compound microscope. When time was not available for immediate detailed study the ovaries were removed and preserved in 5 per cent chloral hydrate for subsequent examination.

As the study progressed, it became evident that four-day intervals were often enough to make dissections, since a period of this duration was required under prevailing conditions to permit marked changes in the developing eggs (fig. 53). This is in agreement with Illingworth[23] in his studies on *Rhagoletis pomonella.*

These dissections of the females showed that the time required under laboratory conditions for the first eggs to be completely developed in the reproductive system was from 10 to 20 days. There was considerable variation in degree of development among individuals of the same age. However, the average for all groups at the seasonal peak of field emergence was 18 days. The higher temperatures with certain limitations increase the rate of egg development, and lower temperatures have a retarding effect.

It is recognized that the conditions of confinement of the flies that were dissected may affect the normal development of the eggs. Back and Pemberton[2] report a relation between kind of food and rate of development of eggs of the melon fly of *Bactrocera cucurbitae* (Coq.). It is entirely possible that lack of certain rays of the solar spectrum together with lack of certain nutritive elements may have affected the flies adversely, resulting in reducing the rate of egg development. An effort was made to compare egg development in the inverted battery-jar cage generally used with that occurring under natural conditions and with that in the small screen cage and in the large cheesecloth cage over walnut trees.

Accordingly an experiment was set up, on the assumption that a 16-mesh wire screen cage over a small bearing Eureka walnut tree would afford natural conditions for the flies. Newly emerged flies were placed in the four different types of cages at the same time. Samples of 10 females from each cage were dissected at 4-day intervals, and the average degree of egg development compared. The first 16 days of the experiment indicated that the large screen cage had not afforded satisfactory environment for the flies, and that the small type of screen cage

was less favorable still. The experiment was duplicated in 1930, with similar results. The average period required for the first eggs to reach maturity in the different types of cages was as follows:

Battery jar, artificial food... 18 days
Large cheesecloth cage over walnut tree, natural food............... 18 days
Large screen cage over walnut tree, natural food..(estimated) 24 days
Small screen cage, artificial food..............................(estimated) 30 days

The egg development of virgin females was studied to a limited extent. Experiments conducted throughout one season, in which 175 virgin females were used, showed that the rate of egg development was approximately the same as that for females confined with males. This conclusion is based on dissection and oviposition data.

In these studies relative to length of preoviposition period, a total of over 1,000 flies were used, of which approximately 500 were dissected to observe egg development. It should be pointed out that many females never deposited any eggs at all, even though apparently the first eggs were fully developed within 12 to 14 days after emergence from the soil.

Oviposition.—The number of eggs produced by a female is a matter of conjecture. Considering the fact that each ovary consists of 24 egg tubes and each egg tube usually contains 4 or 5 developing eggs, the reproductive potential of the species appears to be relatively high. Dissections of females that were known to have deposited over 60 eggs showed no indication of degeneration of the germaria. It seems logical to assume that a female is capable of depositing eggs under favorable conditions as long as she is in good physical condition. Therefore a reliable basis on which to calculate the number of eggs a female may deposit does not exist. It seems probable, however, that under optimum conditions in the field, females may deposit from 200 to 400 eggs.

Experiments were conducted in the field laboratory in 1929 and 1930, in an attempt to obtain detailed data regarding the more important features of oviposition. Series I in 1929 consisted of 12 battery-jar cages in which 2 females and 3 males were confined. Series II in that year consisted of 12 wire-screen cages in which 2 females and 3 males were confined. In 1930, series I and II were battery-jar cages and wire-screen cages, respectively, as before, though 1 female and 2 males were used in series I. In each season all tests were started on the same day, with newly emerged flies. The standard food throughout was lump cane sugar. Moisture was supplied only in the battery-jar cages. Both food and moisture were supplied in the manner previously described. Susceptible host material was supplied by placing a bottle of water containing a twig with two walnuts in each cage. The method of handling

walnuts maintained them in a susceptible condition for a period of 10 to 14 days. However, they were changed weekly in order to supply as favorable condition for oviposition as possible. For brevity, only the data obtained from the 1930 oviposition studies are presented in table 13.

It is evident that battery-jar cages afforded more favorable conditions for oviposition than wire-screen cages. However, the results secured in battery-jar·cages were unsatisfactory. The females were very erratic in oviposition, and some did not oviposit at all, though the average length of life apparently aproached the normal. In battery-jar cages in 1930 the average number of eggs per female was 18.7, while in screen cages it was 1.3. The greatest number of eggs per female was 84 in 7 cavities (cage 14, series I, 1930) and the average length of time between ovipositions was 3 days. In one instance eggs were deposited by flies that were 62 days old, and in a few instances by flies around 50 days old.

Throughout these experiments, particularly in the battery-jar cages, the general behavior of the flies appeared to be normal in all respects except in oviposition. They copulated frequently, which fact led to the suspicion that the females might be voiding their eggs in the cages. Close examination of the interior of the cages at frequent intervals failed to disclose a single egg lying about. On many occasions when the host material was removed from the oviposition record cages it was placed in a stock cage containing many ovipositing females, to determine its susceptibility. In all such tests eggs were deposited in the husk tissue.

The total evidence at hand indicates that under no condition of laboratory confinement employed in this whole biological study of *Rhagoletis completa* have the flies responded normally with respect to oviposition factors. For instance, under natural field conditions the average number of eggs per cavity was 14.9, based on a total of 665 cavities. Under laboratory conditions the average number of eggs from 679 cavities was 8.2. Furthermore only a few instances have been observed under natural conditions where a female made a cavity and failed to deposit eggs in it. This is a fairly common occurrence in the laboratory. In the field laboratory, where large stocks of flies have been kept in battery-jar cages each season for varying periods of time, the average number of cavities per enclosed female has been relatively low.

If the data obtained from oviposition experiments were considered representative, the reproductive potential would be very low. It is believed, however, that the conditions under which the experiments were conducted did not approach the optimum and thereby reduced the fecundity of the females.

TABLE 13

LABORATORY STUDIES ON OVIPOSITION IN 1930

Series I, 1 ♀ + 2 ♂ per battery-jar cage; set up August 8

Age (in days) of ♀ when eggs were deposited — Number of eggs per cavity

Cage No.	10	12	14	16	18	20	22	24	26	28	30	32	34	36	38	40	42	44	46	48	50	52	54	56	62	Average age of females at death, in days	Cavities	Eggs
1		4		7		12	0			9	4															53	6	39
2	0		3						11																	49	3	15
3																										16	0	0
4				13	13	7		7	14														14			61	7	70
5																				9						54	0	0
6																										39	0	0
7															7		10									11	0	0
8	5	0			14						12	7				6										60	8	54
9			0								12					6			10	0						51	0	0
10										6	6															33	2	12
11																										19	0	0
12							3						14				8									45	2	22
13									9																	58	4	25
14		14	12			12		7	13	1		10		17		11				11						55	7	84
15																						1				13	0	0
16		3	0	8	9											2	6		6		11					41	3	11
17				8			7			14															8	67	10	67
18																								3		55	0	0
19																			3			7				34	0	0
20					13																					51	1	13
21										14						14										57	2	10
22		6																	3							44	2	20
23																14	1									52	1	14
24																										6	0	0
25							9		2																	49	3	12
Average																										43	2.4	18.7

TABLE 13—(Concluded)

Series II, 2 ♀ + 3 ♂ per screen cage; set up August 20

Cage No.	Age (in days) of ♀ when eggs were deposited — Number of eggs per cavity																									Average age of females at death, in days	Cavities	Eggs
	10	12	14	16	18	20	22	24	26	28	30	32	34	36	38	40	42	44	46	48	50	52	54	56	62			
1						3									10			2								46	3	15
2																										31	0	0
3									6																	56	1	6
4												4														57	1	4
5																										49	0	0
6																										16	0	0
7																										42	0	0
8																										21	0	0
9											7															34	1	7
10																										45	0	0
11																										32	0	0
12																										38	0	0
Average per ♀																										39	0.3	1.3

Limited observational data indicate that oviposition takes place only during daylight; it may take place at any time during the day when temperature, humidity, and light conditions are favorable. Based on extensive records, oviposition takes place at temperatures from 65° to 103° F. It has never been observed at a relative humidity lower than 60 per cent. Under natural conditions females are usually ovipositing most actively in the late afternoon from about five o'clock until darkness. Exceptions to this occur on dull, overcast days, which are not common during the season of greatest oviposition activity. A relation thus appears to exist between relative humidity and time of oviposition; however, light intensity may also be a regulating factor, judging from the conditions existing when oviposition has been recorded. Under battery-jar cage conditions in the shaded screen laboratory, where the light does not reach normal intensity and the relative humidity is usually 90 per cent or more, oviposition is as commonly observed at midday as at any other time.

When ready to oviposit the female tests the surface of the walnut, apparently to find a suitable place. In doing so she usually goes through a very characteristic set of maneuvers. With proboscis extended she walks about making frequent contacts with this organ. She will often stop and bring the venter of her body to rest on the spot momentarily; sometimes with feet stationary the whole body will be moved quickly up and down, or from one side to the other, or a combination of both movements. When an area is chosen she turns around in a circle several times, often reversing direction, then raises the body well upward and with arched abdomen attempts to force the ovipositor into the tissue at an angle of about 45 degrees. The spot selected frequently proves to be too hard; however, she tests it a few times with the ovipositor before searching for another spot. In the field laboratory one female made four attempts to insert her ovipositor in the same small area of husk without success; another female immediately made several attempts to utilize the same area in vain; a third female followed and with considerable effort finally penetrated the tissue and deposited a batch of eggs. These observations may indicate a variation in muscular strength among individuals.

When a favorable spot is located, the ovipositor is forced into the tissue (fig. 54 *C*) to a depth of about 2 mm and the whole body is moved around in a semicircle, or, often, in a complete circle. This procedure lacerates the tissue below the surface by virtue of the angle assumed by the ovipositor and thereby prepares the cavity for the eggs. The eggs are deposited one at a time. The ovipositor is withdrawn slightly and then forced quickly downward in the nature of a jab. Coincident with

this, the abdomen appears to be contracted to force the egg downward and out the opening of the ovipositor into the cavity. The egg can be detected as it passes the semitransparent portion of the sheath. After each egg is deposited, the ovipositor is withdrawn slightly and usually the body is rotated somewhat to place the next egg in position within the cavity. The eggs are placed one beside another on end at approximately right angles to the surface. The time required for depositing a batch of eggs varies from 3 to 10 minutes. During oviposition the retracted mouth parts pulsate rapidly and continuously.

When flies were confined in cages with walnuts, on several occasions two or three eggs were noted lying on the surface of the walnut near a cavity containing eggs. Apparently the female discharged these eggs directly on the surface. In this connection, with citrus fruits, the females have been observed to discharge their eggs on the surface after unsuccessful attempts to make a cavity (fig. 24, p. 390). In one instance, on peppers, a batch of 18 eggs was observed on the surface near a cavity containing 6 eggs (fig. 27, p. 394). One female is believed to have deposited all of these eggs, continuing to discharge them on the surface after the cavity had been filled.

Usually one female deposits all the eggs contained in a single cavity and does so with one insertion of the ovipositor. In a few exceptional cases out of the hundreds examined in detail, 30 to 40 eggs were present, probably representing two contingents; that is, in approximately half of them the embryo was well developed, while with the remaining ones, practically no development had occurred, which indicated recent deposition. It is not known whether these eggs are the products of one or two females. One female may have deposited both batches at different times; for under laboratory conditions the same individual has been observed to return to an exact spot several days after previous unsuccessful attempts to penetrate the husk tissue for oviposition.

Many observations were recorded of females attempting to oviposit in citrus fruits. With ripe Valencia oranges, for instance, the skin was fairly readily penetrated by the ovipositor, but the tissue below the surface was of such texture that the female could not lacerate it. It was interesting to note how the female would increase the angle of insertion of the ovipositor upon being unable to turn the body around in the original position. The acuteness was usually reduced until the ovipositor was inserted at right angles to the surface, then the female could turn around with ease but without accomplishing the desired purpose. One female worked diligently for half an hour attempting to produce a cavity, then went away and later returned to the same spot and excitedly worked as long again without success. In such cases, however, one egg

was usually placed in the hole made by the ovipositor and others voided on the surface in the immediate vicinity (fig. 24, p. 390). Eggs in such locations dried up before hatching.

The behavior of the flies when confined on walnuts of less susceptible varieties was studied, and data were obtained to supplement field observations. Placentia walnuts were placed in cages with ovipositing flies. In but few instances were the flies capable of penetrating the outer surface of the husk with the ovipositor. In the successful cases observed, penetration was accomplished only after repeated attempts and considerable effort. After the ovipositor had penetrated the outer husk, little difficulty was experienced in lacerating the tissue below the surface. In view of the observations on oranges, where one or more females attempted to use the same puncture for oviposition, it was desirable to know whether or not they would take advantage of artificial punctures on walnuts. Accordingly on several occasions one or more small needle punctures were made on Placentia walnuts in the vicinity of unsuccessful attempts at oviposition by females. They frequently returned to this area to attempt oviposition, but in no instance did they make use of one of the artificial punctures.

Brooks[8] reports that *Rhagoletis suavis* usually takes advantage of abrasions and decaying tissue in ovipositing in walnut husks in eastern United States. Since the biology and habits of *completa* are somewhat similar to those recorded for *suavis,* it was of interest to determine whether or not *completa* would have similar habits, under laboratory conditions. Many records of oviposition in the field showed that *completa* always placed the eggs in healthy tissue, even when natural or artificial abrasions were present for the female to select. Under laboratory conditions Eureka walnuts, with different kinds of abrasions freshly made, and also in various states of decomposition, were placed in cages with ovipositing flies. In many instances the males would take up their vigil beside one of the freshly made artificial punctures, apparently expecting a female to utilize it in oviposition. The female surveyed the abrasions in selecting a spot to insert her eggs, but in no instance did she choose other than healthy tissue. The experiments were repeated many times, with similar results. When walnuts were removed from the cages the abrasions and decaying tissue were carefully examined for the presence of eggs, with negative results.

In one instance males were liberated in a cage containing 25 unmated females, some of which had oviposited previously. A considerable amount of copulation took place and a few batches of eggs were deposited, none of which proved to be fertile. Because of the lateness in the season and the resulting unfavorable condition of host material, the

studies were discontinued before further information was obtained. However, it is expected that fertile eggs would be produced under such conditions. The few batches of infertile eggs may have been deposited by unmated females since the flies were not marked and distinction was not possible.

Signs on the surface of the walnut husk indicating the presence of an egg cavity are very characteristic, both in appearance and location. The actual puncture made by the ovipositor cannot usually be detected with
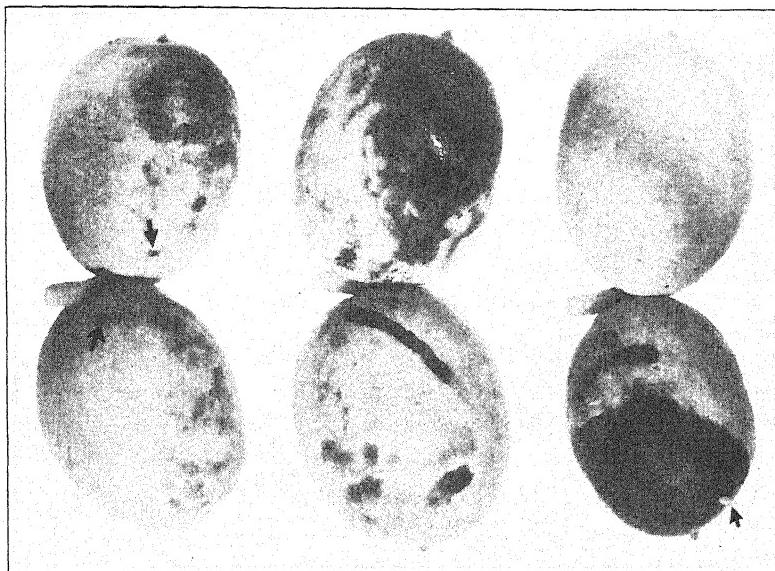
Fig. 55. General appearance of infested nuts in the field. Arrows at left indicate location of egg cavities. Arrow at right points to mature larva emerging from the husk. The so-called "tear stain" is shown on the lower walnuts in center and at right.

the unaided eye; but a droplet of colorless fluid exudes at the point of entry, which very rapidly dries. Flies of both sexes have been observed to imbibe this fluid. The lacerated tissue below the surface readily oxidizes and a resulting circular black spot several millimeters in diameter becomes evident externally (fig. 55). Another common sign a few days after oviposition is the presence of a black "tear stain" effect, resulting from small amounts of exuding husk sap which readily oxidizes after it has mixed with dew and trickled down the surface of the walnut (fig. 55). During the peak of seasonal activity of the fly, when prevailing temperatures are relatively high, the tissue broken down in making the

egg cavity becomes apparent as a dark spot within 1 hour. Later in the season the cavities are not usually detectable until after 12 to 24 hours have elapsed.

When the surface husk tissue over an egg cavity is removed, the eggs are plainly visible. Within several days after the female deposits a batch of eggs, the broken-down inner husk tissue resulting therefrom dries and shrinks, leaving a very perceptible cavity surrounding the eggs. The outline of the cavity is dark, as a result of oxidation, and affords a sharply contrasting background for the pearly-white eggs (fig. 7, p. 371).

For several years, during the peak of oviposition, data were collected regarding the number of egg cavities per walnut, with their respective positions on the surface, and also the number of eggs per cavity. A cavity located in the husk tissue in the anterior one-fourth of the nut was considered in the stem region; when located in the distal one-fourth, in the calyx region; and when located in the remaining central one-half, in the center. The position on the surface was further classified according to the exposure to light. If located so that the female was in the area of most light when ovipositing, often on the upper surface, it was considered outer; when in the area of least light, often on the lower surface, it was considered inner; and when neither of the above conditions prevailed it was considered neutral. The terms "outer" and "inner" were chosen rather than "upper" and "lower" because in many instances a cavity located on the lower surface was actually in position to receive most light, and vice versa. The observations were made on the Eureka variety. The data are presented in table 14.

The data for a 4-year period under field conditions show that 72 per cent of the egg cavities were located in the stem region; 24 per cent in the middle region; and 4 per cent in the calyx region. Under laboratory conditions, in 1931, the percentages are 36, 42, and 22 for the respective regions. Thus a wide difference existed between the relative locations of egg cavities under field and laboratory conditions. The husk-hardness data previously presented (pp. 380–388) show that in 1929 and 1930 the stem region was materially softer than the middle and calyx regions; but that in 1931 negligible differences existed between the hardness of the husk in various regions in Eureka walnuts. The field data for 1928, 1929, and 1930, and the laboratory data for 1931 to a lesser extent, indicate that the flies actually selected the softest regions of the husk in which to oviposit. However, this was not the case in the field in 1931. It is regrettable that detailed data regarding the location of egg cavities under laboratory conditions are not available for the first three seasons, in order to compare with field conditions. While it has been

TABLE 14

LOCATION OF EGG CAVITIES WITH RESPECT TO SURFACE, NUMBER OF EGGS PER CAVITY, AND NUMBER OF EGG CAVITIES PER WALNUT, UNDER FIELD AND LABORATORY CONDITIONS

Year	Total cavities	Total walnuts	Location of egg cavity on surface of walnut (Cavities in per cent of total)						Number of eggs per cavity			Cavities per walnut (Nuts in per cent of total)		
			Stem region	Center region	Calyx region	Outer	Inner	Neutral	Cavities	Total eggs	Eggs per cavity	1	2	3
(Field)														
1928	320	300	70	14	16	…	…	…	194	3,317	17	78	17	5
1929	348	…	60	36	4	12	32	56	116	1,707	15	…	13	1
1930	435	216	74	23	3	…	…	…	228	3,055	13	86	23	5
1931	861	645	76	22	2	13	45	42	127	1,816	14	72	…	…
All years	1,964	1,161	72	24	4	13	41	46	665	9,895	15	76	20	4
(Laboratory) 1931	315	…	36	42	22	…	…	…	679	5,589	8			

Location of cavities with varying number of cavities per nut (field, 1931)

	Total cavities	Total walnuts	Stem region	Center region	Calyx region
1 cavity per nut	462	462	73	25	2
2 cavities per nut	150	150	82	17	1
3 cavities per nut	33	11	70	27	3

shown previously that husk hardness is probably the most important host factor governing oviposition, it cannot be considered the major factor with respect to location of egg cavities, in view of the contradictory field data for 1931. However, extensive field observations regarding oviposition show that undoubtedly husk hardness is a very important factor governing the spot where the eggs are deposited. In most instances observed the females vigorously attempted to insert the ovipositor at least several times in various regions of the husk before succeeding.

Data regarding the location of egg cavities with respect to outer, inner, or neutral positions show that only 13 per cent are placed in the area of most light. Limited husk-hardness data indicate insignificant differences in the hardness of these three locations. The females therefore actually do avoid the outer position for oviposition. Perhaps light intensity is an influencing factor.

The average number of eggs per cavity under field conditions was 14.9, in contrast to 8.2 in the laboratory. In the former instance 99.2 per cent of those cavities examined contained eggs, while in the latter they were present in 88.7 per cent of the cavities. Under field conditions the number of eggs per cavity varied from 4 to 40, while in the laboratory the variation was from 1 to 44 eggs.

Of the 1,161 infested walnuts examined over a 3-year period, 76 per cent exhibited 1 cavity per nut; 20 per cent had 2 cavities; and 4 per cent had 3 cavities. These data were obtained in heavily infested groves (90 per cent or over) where there was maximum opportunity for continued reinfestation. Under laboratory conditions in stock cages containing large numbers of ovipositing females, it was not uncommon to observe from 5 to 10 cavities per walnut, all made within a period of several days. The maximum number recorded per nut was 15. Females probably do not intentionally oviposit in walnuts that are already inhabited by a batch of larvae. Perhaps the repeated sampling on the surface of the nut with the proboscis prior to oviposition, as already described, is for the purpose of ascertaining whether or not larvae are present. In this connection it is of interest to note that after the larvae are about three-fourths mature a characteristic feeding noise is audible. In practically all instances where there were relatively large numbers of egg cavities per walnut under laboratory conditions the eggs were deposited before any larvae hatched.

In the 1930 field control studies, extensive observations were made on a total of 73 trees regarding the location of the infested nuts, that is, whether they occurred on the lower third, or the upper two-thirds of the tree. Of a total of 14,475 nuts examined on the lower portion of the tree,

12.2 per cent were infested. The middle and upper portions were necessarily grouped together, and of a total of 16,778 nuts examined, 17.5 per cent were infested. Therefore it appears that for oviposition the females show a slight preference for those nuts in the middle and upper portions of the tree.

EGG

Incubation.—The incubation period for eggs was determined at the peak of oviposition under field conditions, and also under laboratory conditions for the same period. Under field conditions over 100 cavities were under observation, and complete and detailed information was obtained on 13 cavities, involving a total of 182 eggs. A mean temperature of 73° F, with a range of 52° to 104° F, prevailed for the first 5 days of the period of this study, during which time 85 per cent of the eggs hatched. The average time required for incubation was 120 hours, with a range of from 96 to 240 hours. Fragmentary data involving hundreds of eggs are in general accordance with the relatively few complete detailed records.

The laboratory studies, which yielded fairly accurate data, involved the use of 10 cavities, and a total of 123 eggs. The method used in incubating the eggs under artificial conditions was as follows: Newly deposited eggs were removed from the host tissue and placed on moist filter paper in petri dishes. They were kept darkened except when examined every 6 hours for evidence of hatching. The mean temperature prevailing for the same period of 5 days during which the field studies were made was 81° F, with a range of 75° to 89° F. The average time required for incubation was 72 hours, with a range of 48 to 120 hours. In this experiment many incomplete records likewise support the data obtained from the detailed studies.

Mortality.—In order to determine the mortality occurring naturally with eggs under field conditions, 112 egg cavities were carefully dissected under a binocular microscope. For these studies only nuts possessing one cavity were taken, in order to avoid the possibility of confusing the larvae of two batches of eggs. The eggshells and unhatched eggs were counted and recorded. The husk tissue was carefully dissected in order to determine the number of larvae present. If eggshells were present and none or only a very few larvae were inhabiting the husk, it was assumed that natural enemies had been active. When unhatched eggs were present together with eggshells, and the larvae of the batch were in the third instar, it was assumed that the remaining eggs would not hatch. It was not difficult to distinguish infertile eggs from fertile eggs in which the developing embryo had died. The infertile eggs usually

retained the pearly-white color until they were several weeks old, when they became semitransparent at both ends, their contents then being of watery appearance.

In these field mortality studies a total of 1,239 eggs was involved, of which 244, or 20 per cent, failed to hatch. Counts showed that natural enemies, probably the bug *Triphleps insidiosus* (Say) and the mite *Pediculoides ventricosus* New., or both, were responsible for 40 per cent of this total mortality, or a mortality of 12 per cent of all eggs.

Under artificial incubation conditions in the laboratory, using the method previously described, a total of 636 eggs was studied, of which 141, or 22 per cent, failed to hatch.

LARVA

Hatching.—A few hours before the newly developed larva issues from the egg, movement can be observed. The dark-colored oral hooks and pharyngeal skeleton are very conspicuous through the now transparent chorion. To extricate itself the young larva ruptures the shell by actively scratching a small area on the interior a short distance below the point, and gradually works its body outward through this opening. When the anterior end of the body protrudes through this opening, the oral hooks are used to make firm contacts with the substratum to aid in pulling the body outward. The whole operation usually requires approximately one minute.

Under normal conditions the larva penetrates the host tissue immediately. When hatching under artificial conditions they move about aimlessly and in a few instances have been observed to tunnel between the layers of moistened filter paper. Without food they remain alive from 6 to 12 hours. Locomotion is accomplished through a coördination of the oral hooks, ventral fusiform areas, and the muscles of the individual body segments. The pointed oral hooks normally penetrate the substratum to insure a firm footing while the whole body is drawn forward, each segment being contracted. The ventral fusiform areas make firm contacts while the anterior end of the body is sent forward by the expanding body segments. Thus, essentially, locomotion consists of a series of waves of expansion and contraction of body segments.

Feeding.—The larvae show some tendency toward gregarious habits in feeding. This is not strictly the case, however. Many of the newly hatched larvae leave the egg cavity and enter the normal husk tissue through the same tunnel, but later make individual tunnels. These tunnels often come together and are otherwise usually within close proximity to one another in the particular area of husk tissue inhabited. The developing larvae have no tendency toward scavenger habits, always

showing a preference for healthy tissue for food. When the decaying tissue is all that is available in the area surrounding them, they consume it. This black food in the alimentary canal is plainly visible externally. After the larvae are mature, they are commonly found in a group in the area of broken-down tissue. Under these conditions it is doubtful if they are consuming any appreciable amount of this material for food, since they are usually of creamy-white color. They are probably congregated in this area because it offers less resistance to emergence from the nut for pupation (fig. 56).
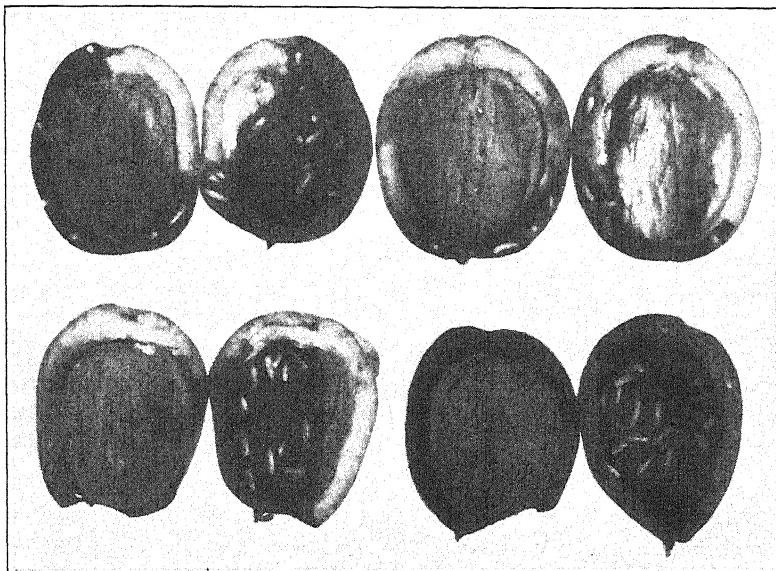
Fig. 56. Infested walnuts sectioned to show larvae *in situ* and characteristic destruction of husk with resultant stained shells.

The amount of food necessary to support a larva to maturity is relatively small. Detailed studies of random samples involving hundreds of walnuts were made in 1930, in which the number of practically mature larvae per nut was determined. The average was 12 larvae per walnut. In one instance, under cage conditions, 167 larvae were recorded from a single walnut. All larvae had attained the third instar, though they were somewhat smaller than normal. Externally the husk was entirely black. The inner husk tissue had been completely destroyed except for a few dry shreds. Other studies were conducted in 1930, to determine the area of stain on the shell of the nut as a measure of the amount of husk consumed. In all cases, except where the entire inner husk is consumed by the larvae, the amount of husk consumed is slightly more than the cor-

responding area of stain on the shell. These data are based on over 1,000 infested nuts and show that 65 per cent of the inhabiting larvae consumed one-half or less of the total inner husk while maturing, and only 14 per cent consumed the entire portion of this tissue.

Development.—Larval metamorphosis essentially consists of two molts or three instars. The methods employed in studying larval development were as follows: Eggs were permitted to hatch under artificial conditions. Sections of green Eureka husks ½ inch square were removed and placed (outer surface down) on moistened filter paper in large petri dishes. A small artificial tunnel was made in the inner tissue and one newly hatched larva placed in the opening. The petri dishes were kept darkened except when the contents were being examined. Over 500 first-instar larvae were used in these studies.

Preliminary observations established the approximate length of time between molts. Beginning 12 hours before the expected molt, samples consisting of 5 larvae were examined at 6-hour intervals to note the change in development. The section of host material was carefully dissected under the binocular microscope in an effort to locate the feeding larva. In many instances, particularly when dealing with the first instar, it was difficult to detect the larva before it was injured in the dissection. When uninjured they were transferred to other sections of fresh host tissue after noting the degree of development. After the first molt they were more readily detected and the mortality due to dissection was considerably reduced. After the second molt it became necessary to use larger sections of host tissue and to transfer the larvae every fourth day until they matured. When the larvae reached approximately three-fourths maturity, the mortality became relatively high again, primarily because of failure to remain in the host tissue. When 50 per cent of the larvae examined had transformed, the average elapsed time was considered an approximation of that required to complete the particular instar. It was necessary to establish an arbitrary criterion because of the variation in time required for larvae of the same batch of eggs to complete an instar. This indicates that they were not behaving normally under artificial conditions. In the field the development of larvae of one batch of eggs appears to be remarkably uniform.

In an attempt to approximate field conditions for purposes of comparison with rates of development under indoor-laboratory conditions, the petri dishes were kept in the standard hygrothermograph shelter in a walnut grove. Thus a record was obtained of the actual temperature conditions to which the larvae were subjected. The data obtained regarding larval development, both indoors and in the field, are summarized in table 15.

General field studies show that an appreciable number of larvae of the early portion of the brood may complete their development in from 18 to 20 days. This information was secured by tagging a large number of nuts in several groves, and also from observations on caged trees. Prevailing temperature conditions and a more succulent host tissue at this time are no doubt responsible for this shortened developmental period.

After the larvae have attained about three-fourths maturity a very characteristic feeding noise can be detected when in a quiet room, or by holding the infested nut near the ear in the field.

TABLE 15

DEVELOPMENT OF LARVAE UNDER ARTIFICIAL CONDITIONS IN THE INDOOR LABORATORY AND IN THE FIELD LABORATORY

	Mean temper- ature	Range	Mean time for development							
			First instar		Second instar		Third instar		Total de- velopment	
	degrees F	*degrees F*	*hours*	*days*	*hours*	*days*	*hours*	*days*	*hours*	*days*
Indoor laboratory	82	67 to 90	127	5.3	199	8.3	343	14.3	669	27.9
Field laboratory	64	45 to 104	233	9.7	312	13.0	338	14.1	883	36.8

Premature Mortality.—Larval mortality during development was fairly accurately determined. At about the peak of larval maturity infested nuts were collected and studied in detail. Data were recorded only from those nuts containing larvae that were practically mature, none having left to pupate. Furthermore, unless the egg cavity or cavities could be definitely located and contents studied, the walnut was discarded. The difference between numbers of empty eggshells, less 12 per cent allowed for the work of natural enemies, and the mature larvae present, was considered to represent the mortality occurring during larval development. Data were obtained from 104 nuts. From 1,610 hatched eggs, 1,209 mature larvae were recorded. The mortality for the lot was 401 larvae, or 24.8 per cent. The average number of mature larvae per nut was 11.6; thus the average mortality was 3.9 larvae per nut.

Emergence from Walnuts.—When the larvae reach maturity they usually tend to congregate in the area of broken-down inner husk tissue, where they remain for a short while before emerging. In some instances this area includes the cavity that contained the eggs from which the

larvae originated. In such cases the puncture made by the female for oviposition is usually taken advantage of by the larvae in their exit. The size of the original opening is too small to detect with the unaided eye, but subsequent degeneration of the tissue often results in an opening from ½ to 1 millimeter in diameter. In any case it usually serves as a basis for the exit hole since it is fairly readily enlarged by rupturing the surrounding outer husk tissue. This tissue is now usually of a thin leathery texture. If an opening does not already exist, the larvae apparently are capable of making one with their oral hooks. In no instance has any evidence been found of larvae having emerged from a walnut through healthy tissue.

All larvae of one batch of eggs usually emerge through the same small opening. The oral hooks are placed through the opening to make contact on the outer surface. This serves to draw the first few body segments through the opening until the size of the hole prevents further ready penetration. The outward-projecting anterior portion of the body is then rotated in as wide an arc as possible, which serves to draw a portion of the plastic body segment through the hole at an angle. The emerging larva seems to force the body contents forward into the projecting anterior end as much as possible to aid in the exit. From 3 to 15 minutes is usually required for a larva to extricate itself completely from a nut under these conditions. When finally freed, it drops to the ground. All larvae within a single husk do not emerge one immediately following the other. In fact several days may elapse after the first larva emerges before all larvae have departed from the nut, particularly when only one exit hole is utilized.

In some instances when the uninjured portion of the husk of an infested walnut opens characteristically in ripening, the mature larvae leave through the split in the husk. This occurs only when the split in the uninjured husk is very close to injured tissue, which leaves a direct opening to the outside.

In the regular harvesting process the trees are systematically shaken and the infested nuts generally drop with husks adhering. The husk usually splits as a result of the impact with the ground, particularly if the nut contains mature larvae. Under these conditions the larvae emerge at about the same time and enter the soil immediately. It commonly happens that, because of the decaying husk tissue around the stem region, the walnut with husk attached pulls away from the stem at this point, the stem remaining on the tree. This results in a fairly large opening through which the larvae emerge if the husk is not ruptured in their immediate vicinity. Because of the possible bearing on control measures, an effort was made to determine the approximate percentage

of nuts from which the larvae had emerged at time of harvest for the Eureka variety of walnut. The data obtained are summarized in table 16.

The time of harvest in 1929 and in 1932 was earlier than that for 1930 and 1931. Furthermore it was considerably earlier in 1932 than in 1929. The data clearly show that the earlier the harvest the greater the number of walnuts that still contain larvae.

Occasionally the injured husk dries and hardens around one or more larvae, thereby trapping them. Since larvae are capable of pupating shortly after the third instar is reached, they usually enter the pupal stage under such conditions. In certain detailed studies involving an

TABLE 16

SEASONAL COMPARISON OF NUMBER OF INFESTED WALNUTS FROM WHICH LARVAL EMERGENCE HAD OCCURRED WHEN HARVEST BEGAN

Year	Date	Total walnuts examined	Per cent of walnuts from which larvae had emerged
1929	October 4	1,124	43
1930	October 15	2,408	77
1931	October 10	1,818	87
1932	September 25	2,790	25

examination of every nut on a medium-sized infested tree, together with the larvae and pupae present in the nuts and in the soil, it was found that 0.14 per cent of the total pupae occurred beneath the dried husk, where the larvae had been trapped and had pupated.

Under natural conditions in the field, larvae have been observed to emerge from the nuts at all hours of the day. However, they were more commonly noted emerging in the cooler portions of the day. In several instances larvae were observed to drop onto the soil in direct sunlight during midday, where they were killed by the high temperature before they could enter the soil. This suggested the possibility of a relation between larval emergence and time of day with specific reference to temperature. An experiment was conducted in an attempt to obtain information relating to this matter. The soil beneath two medium-sized trees in a heavily infested grove was covered with canvas, in order to collect the larvae periodically as they dropped from the nuts. These records, together with prevailing air temperature and humidity, are presented in table 17.

The greater percentage of larvae emerged in the morning, between the hours of 5:30 and 8:00 o'clock, on both days. Practically the entire emergence on both days occurred when the temperature range was from

40° to 71° F, with the range of relative humidity from 95 to 40 per cent. Larvae did not emerge during the night, although the temperature and relative humidity apparently were not unfavorable. Throughout the greater part of both nights the temperature was not below 45 degrees nor the relative humidity below 80 per cent. It seems probable that tem-

TABLE 17

TIME OF DAY WHEN LARVAE EMERGED FROM WALNUTS; 1931

Hour of day	Tempera-ture, degrees F	Relative humidity, in per cent	Tree No. 1	Tree No. 2	Both trees	Per cent of total larvae per day
			Number of larvae			
September 21						
Before 5 a.m.	46	87	0	0	0	0.0
5– 6 a.m.	47	80	195	270	465	33.4
6– 7 a.m.	58	56	284	289	573	41.2
7– 8 a.m.	71	40	160	113	273	19.6
8– 9 a.m.	80	35	26	14	40	2.9
9–10 a.m.	82	28	10	7	17	1.2
10–11 a.m.	87	24	3	7	10	0.7
11 a.m.–12 m.	88	23	1	2	3	0.2
12 m.–1 p.m.	90	22	1	6	7	0.5
1–3 p.m.	85	26	2	2	4	0.3
3–5 p.m.	77	38	0	0	0	0.0
Total	....	....	682	710	1,392	100.0
September 22						
Before 5 a.m.	41	95	0	0	0	0.0
5– 6 a.m.	40	94	85	55	140	7.7
6– 7 a.m.	49	69	295	310	605	33.4
7– 8 a.m.	58	50	400	290	690	38.1
8– 9 a.m.	66	42	136	238	374	20.6
9–10 a.m.	74	36	0	0	0	0.0
10 a.m.–5 p.m.	71	60	0	0	0	0.0
Total	....	....	916	893	1,809	100.0

perature would bear more of a relation to time of larval emergence than would humidity; and, if true, the limits of favorable temperature are fairly narrow.

Tropic Responses of the Larva.—Mature larvae show marked positive geotropism. This is an important factor in the preservation of the species. Under favorable conditions in light or darkness they disappear below the surface of the soil within 1 to 5 minutes after dropping thereon from their host. Their downward migration is not always perpendicular, for they appear to take advantage of the path of least resistance within

certain limits. Larvae in the second instar, and in the early portion of the third instar, do not exhibit such a marked geotropic response as do mature larvae.

Larvae in all stages appear to be positively thigmotropic, though to a limited degree. Perhaps it is in reality a geotropic response, for the conditions of the observations were not such that a fine distinction could be made. Whenever larvae were contained in petri dishes in which they were originally placed on moistened filter paper, approximately 25 per cent of them would find their way between and beneath the sections of paper within 24 hours. The behavior was similar in darkness and in daylight.

A simple method was employed to obtain information relative to the phototropic reactions of larvae. A wooden tray, 18 inches square and 2½ inches deep, was prepared as follows: One-half of the tray was lined with black cloth and also covered with the same material; the remaining half was lined with white cloth and left without a covering. With the tray orientated so that the black end was northward, lots consisting of 100 mature larvae were placed in the center of the tray within the confines of a small circle drawn with pencil. In this way one-half of the larvae were placed on the black cloth, just within the edge of the darkened end, the others being on white cloth and in daylight. Six hundred larvae were used in these tests. The number of larvae in each end of the tray was counted after they had dispersed from the center. An average of 81 per cent migrated to the darkened end, and the remaining 19 per cent moved about in daylight. After the limit of the darkened end had been reached, many of the larvae wandered aimlessly about, often returning to the daylight end. This test indicates that mature larvae are inclined toward negative phototropism.

A similar test was conducted in a regular photographic dark room, except that soil was placed in the bottom of the tray and a 50-watt electric light centrally located above the tray provided the only source of illumination. It was thought that if the larvae exhibited negative phototropism under these conditions they would enter the soil after migrating into the darkened end, and vice versa. Two hundred mature larvae were liberated in the center of the tray on the soil. There was very little migration in any direction except downward. After pupation, the soil was sifted in sections and 57 per cent of the pupae were taken in the soil of the dark end, while the remaining 43 per cent were in the light end. Under the conditions of this test, the positive geotropic response was probably so great that any significant tendencies toward phototropic responses were masked. However, burrowing into the soil may have been a negative phototropic response.

Entering Soil.—When the mature larvae come into contact with soil, they begin to burrow downward immediately, or as soon as a crack or crevice is found in which to obtain a firm hold with the mouth hooks. Penetration is effected in much the same manner as in migration on smooth surfaces. Occasionally a larva has been observed to enter the soil to a depth of about one-half its length, and when further penetration appeared difficult, to rotate the protruding posterior end, inscribing an arc in the same manner as with the anterior end when emerging from a small hole in a walnut husk.

TABLE 18

DEPTH TO WHICH LARVAE NORMALLY ENTER ORCHARD SOIL TO PUPATE

Depth, in inches	Grove A Hanford fine sandy loam		Grove B Yolo clay loam	
	Pupae*	Per cent of total	Pupae†	Per cent of total
1	1,286	74.0	54	12.0
2	371	21.0	118	26.0
3	57	3.0	207	46.0
4	19	1.0	58	13.0
5	7	0.4	13	3.0
6	1	0.1	3	0.7
7	1	0.1	1	0.2
8	0	0.0	0	0.0
9	0	0.0	0	0.0
Total	1,742	100.0	454	100 0

* Eight plots of 1 sq. ft. each.
† Four plots of 1 sq. ft. each.

The loamy types of soil in the infested area under normal tillage at harvest time are readily penetrated by the larvae. These same soils may be packed by hand to such an extent that larvae are unable to penetrate them. Extensive sifting tests were made to determine the depth that larvae penetrate orchard soil under natural conditions to pupate. One square foot of soil in various locations was carefully excavated in layers 1 inch in thickness and sifted, and the number of pupae contained in each layer recorded. A total of 8 square feet of soil was sifted in grove A, and 4 square feet of soil in grove B. These data are presented in table 18.

In grove A the greater percentage of larvae did not penetrate the soil deeper than 1 inch; while in grove B the greatest percentage of larvae were found at the 3-inch level. Aside from difference in soil type, the surface soil in grove B was loose and very dry at the time of larval emergence from the walnuts. In both groves a few larvae reached the depth of 7 inches before pupating.

Within the infested area walnuts are found growing in five different types of soil: Hanford fine sandy loam, Hanford fine sand, Hanford sand, Yolo clay loam, and Chino clay adobe. It was desirable to study the effect of soil type on the larvae with respect to relative ease of penetration, depth of penetration, and mortality after penetration but before pupation. Samples of these types of soils were collected, sifted, and packed in a standard manner to a depth of 9 inches in battery jars. Sifting of the Chino clay adobe was not practical; samples were therefore taken from a bare area adjoining a walnut grove. An effort was made to maintain the samples practically comparable with respect to moisture present. All tests were set up in duplicate. One hundred and fifty mature larvae taken directly from host tissue were dropped onto the surface of the soil in each jar. The jars were arranged on a table under a large walnut tree and were not moved after the larvae were placed on the soil. A section of plate glass was used to cover the jars, to prevent other larvae from dropping into the jars and also to conserve the moisture present.

Within 2 hours after the experiment was set up, 95 per cent of the larvae had entered all types of soil except the Hanford sand. Here they moved around a great deal on the surface without being able to effect penetration. The sand shifted with them and the greater percentage of the larvae were unable to go downward. When this was observed, another jar of Hanford sand was set up and wetted until penetration by water had taken place to a depth of 6 inches. One hundred larvae were placed on the surface and within 1 hour all but three larvae had disappeared. Water causes the sand to cohere sufficiently for the larvae to make the firm contact necessary to force their bodies downward. The results of the penetration experiments are summarized in table 19.

Hanford sand was the only soil type that materially interfered with normal larval penetration. Furthermore in this soil mortality after pupation was very high. The explanation for this fact is lacking; perhaps weakening of the larvae by the several days of continuous activity in attempting to enter the sand partly accounts for it. The following experiment furnishes contributory evidence regarding this matter: Orchard soil was sifted and packed in a standard manner in a battery jar. In another jar the procedure was identical, except for further packing of the surface with the bottom of a bottle. This left the surface fairly hard. One hundred and seventy-five larvae were dropped on the soil surface in each jar. In the first jar they penetrated the soil within an hour; while in the second jar none was successful. However, in the second jar 9 per cent of the larvae had pupated on the surface within 24 hours, and 93 per cent within 68 hours. The larval mortality was 7

TABLE 19

RELATION OF CERTAIN TYPES OF SOIL TO LARVAL PENETRATION

Soil type	Test number	Mortality on surface, per cent	Pupated on surface, per cent	Per cent of total larvae that reached various depths in soil							Total pupae		Mortality in soil before pupation, in per cent
				0-½ inch	½-1 inch	1-1½ inches	1½-2 inches	2-2½ inches	2½-3 inches	3-3½ inches	Number	Per cent	
Hanford fine sandy loam	1	2	0.0	0.0	15.0	29.0	28.0	12.0	6.0	0.7	137	91	7.00
	1a	2	1.0	3.0	12.0	28.0	37.0	0.0	0.0	0.0	123	82	15.00
Hanford fine sand	2	1	0.7	0.7	4.0	28.0	45.0	15.0	2.0	0.0	144	96	0.01
	2a	0	0.0	1.0	2.0	19.0	41.0	19.0	9.0	1.0	137	91	0.10
Hanford sand	3	44	55.0*	0.0	0.0	0.7	0.0	0.0	0.0	0.0	84	56	0.00
	3a	38	59.0†	0.0	1.0	0.0	0.0	0.0	1.0	0.0	93	62	0.00
Yolo clay loam	4	1	1.0	1.0	12.0	53.0	20.0	4.0	1.0	0.0	140	93	0.04
	4a	0	0.0	0.7	13.0	26.0	55.0	2.0	0.7	0.0	146	97	0.03
Chino clay adobe	5	0	0.7	4.0	33.0	22.0	0.7	0.0	0.0	0.0	90‡	60	40.00
	5a	2	1.0	14.0	28.0	12.0	0.7	0.0	0.0	0.0	84‡	56	42.00

* Eighty-two individuals dead, 1 alive.
† Perfectly formed puparia, though insects dead within.
‡ A few pupae were undoubtedly not accounted for, because of inability to pulverize all soil completely.

per cent. Practically all of the pupae were alive and apparently normal. In this test the larvae moved around on the surface attempting to enter, but less energy seemed to be required than when migrating on sand that was constantly shifting under them.

Chino clay adobe is a very heavy type of soil, and naturally offers great resistance to penetration. This fact may account for the relative shallowness of penetration and the increased mortality in comparison with the other three more favorable types of soil. In this adobe soil, the greater percentage of larvae penetrated to a depth of from 1½ to 2 inches.

Laboratory Studies Regarding Effect of Temperature Upon the Mature Larva.—Preliminary laboratory experiments were conducted to determine the effect of temperature upon the mature larva. For each individual experiment, 100 larvae were taken directly from walnut husks when the test was begun. Petri dishes 3½ inches in diameter and ½ inch deep, with several pieces of filter paper placed in the lower section, were used for containers. At the beginning of each test the filter paper was saturated with distilled water. In certain tests this moist condition was maintained throughout the experiment, while in others the paper was not moistened again during the period of the particular test. Temperature was maintained fairly constant though the equipment available did not permit entirely satisfactory control. A summary of the details of these experiments and the results obtained are presented in table 20.

At 30° F the exposure varied from 35 hours to 110 hours and in all instances mortality was materially increased over that in the controls. However, there was no consistent relation between length of exposure and mortality. In all instances after exposure to a temperature of 30° F, when removed and placed at the same temperature as the control (72° F), pupation was materially stimulated as compared with the control. However, a high percentage of the larvae died within the puparium shortly after it was formed.

Considering the total elapsed time, there was no significant difference in larval mortality between the tests at 36° and 42° F and the control at 72° F. At 50° F, after 150 hours' exposure, pupation was delayed somewhat, though mortality was approximately equivalent to that of the control. At this same temperature, after 800 hours' exposure, mortality was high shortly after pupation.

At 72° F (control) in the dry container, mortality was very high and consequently few larvae pupated. In the wet container no larvae pupated before 100 hours' exposure; after 300 hours' exposure there were 14 per cent live larvae, 12 per cent dead larvae, 62 per cent live pupae,

TABLE 20
Effect of Temperature Upon Mature Larvae

Experiment No.*	Moisture condition in container	Temperature	Exposure	24 hours			48 hours			100 hours			150 hours			Remarks
				Mortality, larvae	Pupated		Mortality, larvae	Pupated		Mortality, larvae	Pupated		Mortality, larvae	Pupated		
					Alive	Mortality		Alive	Mortality		Alive	Mortality		Alive	Mortality	
		degrees F	hours	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	
A	Moist‡	30	35	8	12	0	8	20	16	20	32	16	28	28	20	Larvae frozen stiff after 24 hours' exposure
A1	Moist		45	40	8	4	44	12	8	52	4	16	28	30	...	
A2	Dry‖		55	14	2	0	40	6	0	44	4	2	92	2	4	
A2a	Moist		55	16	10	0	32	20	8	48	22	12	66	4	30	
A3	Moist		72	0	8	0	24	32	8	36	32	12	48	20	32	
A4	Moist		110	0	0	0	24	0	0	40	0	0	72	8	0	
B	Moist	36	110	2	2	0	4	14	0	4	20	0	6	34	0	Larvae motionless at this temperature
B1	Moist		135	0	0	0	2	0	0	4	10	0	16	30	0	
C	Moist	42	150	2	12	0	2	12	0	4	18	0	10	24	0	Larvae move very slowly. Forty-two per cent pupated in chamber after 775 hours' exposure
C1	Moist		800	6	50	0	10	54	20	18	42	26	18	38	44	
D	Moist	50	†	0	0	0	0	0	0	4	0	0	4	0	0	Larvae move slowly. Six per cent dead and 24 per cent pupated in chamber after 550 hours' exposure
D1	Moist		800	8	42	22	...	...	...	...	...	...	30	36	34	

Time at 72° F after removal from experimental temperature chamber: or length of exposure†

* 100 larvae in each experiment.

† Heading "Length of Exposure," applies to experiments *D, E, E1, F, F1, G, G1.*

‡ "Moist" indicates that water was applied as required to maintain a fairly constant amount of moisture inside petri dish.

‖ "Dry" indicates that no water was applied to filter paper inside petri dishes after experiment was begun.

TABLE 20—(Concluded)

Experiment No.*	Moisture condition in container	Temperature	Exposure	24 hours			48 hours			100 hours			150 hours			Remarks
				Mortality, larvae	Pupated Alive	Mortality	Mortality, larvae	Pupated Alive	Mortality	Mortality, larvae	Pupated Alive	Mortality	Mortality, larvae	Pupated Alive	Mortality	
		degrees F	hours	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	
E (Controls)	Dry‖	72	†	2	0	0	8	0	0	12	0	0	12	8	0	Eighty-eight per cent dead larvae. Six per cent dead pupae, and 6 per cent live pupae after 250 hours
E1	Moist‡		†	4	0	0	4	0	0	4	18	0	4	24	0	Twelve per cent dead larvae, 12 per cent dead pupae, and 62 per cent live pupae after 300 hours
F	Dry	90	†	24	0	0	34	0	0	100	0	0	…	…	…	Larvae move actively and constantly
F1	Moist		†	0	0	0	0	0	0	10	4	0	30	18	0	
G	Dry	100	†	14	0	0	92	0	0	100	0	0	…	…	…	Larvae move very actively and constantly
G1	Moist		†	2	0	0	40	0	0	100	0	0	…	…	…	
H	Moist	115	½	8	0	…	12	2	0	20	10	0	52	16	0	Larvae move very actively and constantly
H1			¾	100¶	…	…	…	…	…	…	…	…	…	…	…	
I	Moist	125	½	84	…	…	92	…	…	94	0	0	100	0	0	Larvae move very actively and constantly
I1			¾	100¶	…	…	…	…	…	…	…	…	…	…	…	

* 100 larvae in each experiment.

† Heading "Length of Exposure," applies to experiments D, E, E1, F, F1, G, G1.

‡ "Moist" indicates that water was applied as required to maintain a fairly constant amount of moisture inside petri dish.

‖ "Dry" indicates that no water was applied to filter paper inside petri dishes after experiment was begun.

¶ Dead at end of exposure.

and 12 per cent dead pupae. Thus these conditions failed to stimulate pupation, for under field conditions when larvae were forced to pupate on the soil surface they did so in from 24 to 70 hours.

At temperatures of 90° to 100° F the mortality was very high and in proportion to the temperature and length of exposure. A relatively small percentage of larvae pupated at 90° F in moist tests while none pupated in the dry tests, nor in either the moist or dry tests at 100° F. Temperatures of 115° and 125° F for ½ hour were fatal to a high percentage of the larvae, while at a ¾-hour exposure, all larvae were killed. All temperatures between 90° and 125° F to which larvae were exposed greatly stimulated activity, and in these tests the larvae were in motion constantly.

PUPA

Time Required for Various Stages.—The following experiment was conducted in an effort to obtain desired data on pupation. At the peak of larval emergence from the nuts, 12 battery jars were filled to a depth of 3 inches with sifted top soil from an orchard. The soil was packed in a standard manner and the battery jars were then buried to a depth of 3 inches in the soil surrounding a walnut tree. This arrangement very closely approached orchard conditions. One hundred mature larvae were taken directly from host tissue and were dropped onto the soil in each container, which was then covered with cheesecloth. In order to determine the necessary time for the puparia to be formed, single jars were removed at 6-hour intervals and the soil sifted. If puparia had not formed, the material was discarded after noting the degree of development. This was necessary in order to eliminate possible error by disturbing the larvae before they became completely inactive. Within 12 hours a few larvae were beginning to contract in length, assume a straw color, and otherwise show indications of the formation of the puparium. Within 18 hours, approximately 75 per cent of the larvae were in this condition; and in 24 hours the puparium was completely formed in practically all instances.

In entering the pupal stage the plastic larval skin hardens. This phenomenon takes place progressively from the posterior end forward. A decided straw color becomes evident with the hardening. The segments of the larvae are contracted and the anterior three segments are telescoped into the fourth. This leaves the anterior spiracles projecting slightly forward.

After the time required for formation of the puparium was determined, the subsequent morphologic changes were studied. The time required under field conditions for the consummation of these various

changes was also determined. Batches of 50 puparia of known age were carefully dissected at 6-hour intervals and the stage of development recorded. These records were tabulated and the development described, employing the terminology used by Snodgrass[36] in his study of the anatomy and metamorphosis of *Rhagoletis pomonella*. Within 36 hours after the formation of the puparium, there is an additional larval molt. The larva is enclosed within a hard shell and consequently remains stationary. This condition is commonly referred to as the prepupal stage. Histologic and morphologic changes continue, and within 90 to 100 hours after the puparium is formed the insect is in the early phase of the cryptocephalic stage. At this time small rudiments of the developing appendages are evident, though the future head is not visible. Within 10 to 20 hours more the final phase of the cryptocephalic stage has been reached. The rudiments of the appendages are larger than in the early phase, the abdomen still retains its prepupal form, no head is visible, and the prepupal skin has been shed over the entire body.

After 10 to 20 hours more have elapsed, the early phase of the phanerocephalic stage is reached. The head is everted though relatively small in size, the appendages are of increased size, and the abdomen still retains its prepupal form. The second phase of the phanerocephalic stage, or the final pupal stage, is attained within 10 to 12 hours more. The general form of the adult body is recognizable. The head is very large and the appendages are about full length, extending nearly to the end of the abdomen. Thus under the prevailing field conditions, which were fairly representative, the true pupal stage (fig. 11, p. 374) was reached within 145 to 175 hours after the larva entered the soil.

Population of Pupae in Soil.—In 1929, in this same grove just prior to the beginning of emergence, representative areas of soil were sifted under two trees in order to estimate the total number of pupae present. There was an average of 26 pupae per square foot of soil under tree No. 1, and the total area directly beneath the tree was 1,134 square feet. Thus the calculated total number of pupae was 29,484. Under tree No. 2 there was an average of 31 pupae per square foot and the total area was 1,385 square feet. Thus the calculated number of pupae present was 42,935.

Soil Temperature as a Mortality Factor.—In adult emergence studies of 1930, an attempt was made to determine the effect on time and rate of emergence when pupae were under natural conditions in soil in cages exposed to total sunlight in contrast to partial shade. Accordingly, in the fall of 1929, areas for emergence cages were seeded after the method described previously. Two cages were in total sunlight in the grove, while the other two were in the usual locations under trees in partial

TABLE 21

MORTALITY OF PUPAE AT VARYING DEPTHS IN SOIL UNDER TOTAL SUNLIGHT AND PARTIALLY SHADED CONDITIONS

Cage No.	Location	State of pupae	Per cent of total pupae at various depths in soil						Total	
			0-1 inch	1-2 inches	2-3 inches	3-4 inches	4-5 inches	5-6 inches	Number	Per cent
8	Total sunlight	Alive	0.0	1.0	1.0	0.2	0.4	0.0	13	3
		Dead	61.0	22.0	10.0	3.0	2.0	0.2	478	97
8a	Total sunlight	Alive	0.0	0.0	8.0	3.0	0.0	0.0	15	11
		Dead	24.0	34.0	27.0	4.0	0.7	0.0	127	89
9	Total shade	Alive	0.4	12.0	43.0	11.0	3.0	1.0	197	69
		Dead	0.4	10.0	17.0	4.0	0.7	0.0	88	31
9a	Partial shade	Alive	0.0	16.0	34.0	2.0	9.0	2.0	63	65
		Dead	9.0	14.0	7.0	2.0	1.0	1.0	34	35

shade. When seasonal adult emergence began in 1930, the cages in total sunlight yielded no flies. Several weeks after emergence had begun, a few of the pupae in the soil of these two cages were examined and found to be dead. It was then desirable to obtain more detailed information. A total of 4 square feet of soil in different locations in each cage was carefully excavated in layers of 1 inch in thickness, and sifted. The pupae obtained were determined to be alive or dead by crushing, which is a reliable test. Data resulting from these excavations are presented in

TABLE 22

DISTRIBUTION OF PUPAE WITH RESPECT TO DEPTH IN ORCHARD SOIL DUE TO CULTIVATION PRACTICES

Lot No.	Number of pupae per sq. ft. at various depths in soil													
	Top	0-1 in.	1-2 in.	2-3 in.	3-4 in.	4-5 in.	5-6 in.	6-7 in.	7-8 in.	8-9 in.	9-10 in.	10-11 in.	11-12 in.	12-14 in.
1	0	3	5	1	3	2	·2	1	0	1	0	0	0	0
2	0	4	2	2	4	7	6	3	5	3	2	4	0	0
3	1	0	2	1	0	0	4	3	0	0	0	0	0	0
4	0	6	2	1	3	10	2	10	5	2	0	2	0	0
5	0	1	3	7	3	11	7	0	5	1	1	1	3	0
6	0	0	0	2	0	0	0	0	0	0	0	0	0	0
7	0	0	0	3	1	3	0	2	1	1	0	0	0	0
8	1	1	3	2	5	1	1	6	6	3	7	2	1	0
9	0	4	0	7	2 ·	3	1	5	2	2	0	1	3	0
10	0	2	1	4	7	0	1	2	3	2	0	0	3	0
Total	2	21	18	30	28	37	24	32	27	15	10	10	10	264
Per cent	*1*	*8*	*7*	*11*	*11*	*14*	*9*	*12*	*10*	*6*	*4*	*4*	*4*	*100*

table 21. The data indicate that apparently a high mortality results from high soil temperatures when the pupae remain undisturbed in the soil in total sunlight.

Normal orchard-cultivation practices alter the situation somewhat. In late June, 1928, after the last cultivation of the soil, before emergence began and before soil temperatures became high, the relative distribution of pupae with respect to depth was studied. The grove in which the studies were made is the same one referred to as grove A in table 18 and also the one from which the data presented in table 22 were obtained. One square foot of soil was sifted from each of ten locations within the grove. Strata 1 inch in thickness were sifted individually. The results are presented in table 22.

As a result of cultivation more than half of the pupae were fairly evenly distributed at depths of from 3 to 8 inches, while an appreciable number were found at a depth of 12 inches. Thus cultivation of the soil serves to decrease environmental resistance considerably.

Moisture and Dryness as Mortality Factors.—The only information available regarding the effect of moisture and dryness on pupal mortality is forthcoming from general observations over a period of years. Occasionally an "indicator" adult emergence cage was located on soil that was very wet for periods of weeks at a time when the pupae were in the soil. At the other extreme, cages were sometimes inadvertently placed on very dry soil. Although detailed records of emergence from these indicator cages were not kept, many flies emerged, which demonstrates that neither the condition of excess moisture nor that of dryness was fatal to all of the pupae. In many instances adults have emerged from pupae kept indoors in glass vials without soil for a year. In one instance, under these conditions, adults emerged two years after the larvae pupated.

Summary of Pupal Mortality.—The extent of pupal mortality was fairly well determined over a period of three years for pupae of varying

TABLE 23

MORTALITY OF PUPAE UNDER NATURAL CONDITIONS*

Year	Age of pupae	Total number pupae	Mortality
	years		*per cent*
1929	1	2,000	39
1930	1	2,000	51
	2	1,400	33
	3	35	17
1931	1	2,000	23
	2	657	56
	3	54	85
	4	5	60

*Pupae buried in appropriate containers at depth of 5 inches under walnut trees.

age. Normal pupae of known age were used in these studies. In all instances the pupae of different ages were kept segregated. The data obtained from these studies are presented in table 23. It is evident that the percentage of mortality varies greatly among pupae of different ages and in different seasons.

Dormancy.—The nature of dormancy, or the diapause phenomenon, in *Rhagoletis completa* is very interesting though apparently complicated. A brief treatment of this phenomenon in insects was published in 1931,[6] in which reference to *R. completa* was made and certain limited data presented. However, since that time more information has accumulated and the total data have been reworked. The data presented

previously in the treatment of various factors affecting adult emergence (pp. 404–425) indicate that under natural conditions temperature is a very important physical factor relating to the matter.

To summarize briefly, the calculated seasonal peak of emergence was reached approximately 27 days earlier, and the percentage of annual-generation flies was increased approximately 26 per cent after the mild winters over that after cold winters. Also the time of emergence was earlier in those cases where the soil received the greatest heat from sunlight throughout the dormant period of annual-generation individuals. However, the exact relation of temperature to dormancy is not known beyond circumstantial evidence, which indicates that a certain minimum amount of heat units supplied by a range of fluctuating temperatures seem to be required for the termination of dormancy. Under fairly constant temperature conditions in the laboratory, dormancy was not broken to any appreciable extent when the equivalent number of total day-degrees temperature apparently required under field conditions had elapsed. It seems probable that in some manner temperature, together with unknown genetical factors, regulates the length of the dormant period in multi-annual-generation individuals. A summary of certain features pertaining to dormancy has already been presented in table 2 (p. 420).

Limited studies were conducted to determine the effect upon dormancy of varying lengths of exposure to temperatures of 20° and 30° F. One hundred pupae were used in each experiment. All experiments were begun on the same day. Pupae 20 days old were taken directly from soil and placed in moist sand in glass containers in which they were subjected to the several temperature conditions. The control lot was kept in an indoor insectary, and likewise all other lots at the termination of the respective periods of exposure to low temperatures. The data obtained are presented in table 24.

In only a few instances was there any indication that dormancy was materially altered by the treatments. In tests 3, 4, 5, 6, and 8 there were material increases in percentage of total emergence at the end of 7 months, when compared with the control. In these same tests, with the exception of test 6, increases in percentage of total emergence were yet evident at the end of 12 months, in comparison with the control. In general, the mortality at the end of 12 months was slightly less in the treated series than in the control. Repeated tests are necessary before important conclusions are warranted.

Preliminary experiments were conducted in an effort to determine the effect of certain chemicals in terminating the diapause. Only a few of the chemicals that have given positive results in breaking the dor-

mancy of plants were tested. In each test 200 pupae of the same age were
used. Random concentrations of the materials were used, with several
variations. Treatment consisted of soaking the pupae for varying
lengths of time in the liquid, or subjecting them to an atmosphere of gas
in certain instances. After treatment each lot was segregated in moist
sand in containers that were kept indoors under heated insectary con-
ditions. Adequate controls were maintained. Emergence of flies was re-

TABLE 24

EFFECT OF TEMPERATURE UPON DORMANCY OF PUPAE*

Experiment No.	Temperature	Exposure	Emergence		Mortality	Pupae alive
			7 mos.	12 mos.	12 mos.	12 mos.
			Per cent of total pupae			
	degrees F	*hours*				
1		33	0	22	50	28
2		47	5	19	57	24
3	20	154	10	48	38	14
4		240	14	47	26	27
5		336	11	46	43	11
6		792	24	31	63	6
7		312	4	42	19	39
8	30	528	40	50	39	11
9		1,084	8	28	54	18
10	73	Continuous control	4	36	51	13

* 100 pupae were used in each experiment.

corded at weekly intervals. The materials and concentrations used and
length of exposure, together with results obtained, are summarized in
table 25.

Potassium thiocyanate apparently produced a slight effect upon dor-
mancy, since an appreciable percentage emerged within 138 days after
treatment. The mortality, however, was materially higher than in com-
parable controls after 335 days. Thiourea appeared to stimulate emer-
gence somewhat after 130 days without any effect upon the mortality.
Ethylene chlorohydrin as gas, ethylene dichloride, carbon bisfulfide,
hydrocyanic acid gas, and xylene were fatal to the pupae under the
conditions tested. The results obtained in these studies, particularly
with potassium thiocyanate and thiourea, though inadequate to base con-
clusions upon, suggest that these and other chemicals might profitably
be employed in further studies of the nature of dormancy in insects.

TABLE 25
EFFECT OF CERTAIN CHEMICALS UPON DORMANCY OF PUPAE*

Material	Experiment No.	Concentration — Liquid, per cent	Concentration — Gas, cc vaporized per 10,000 cc space	Exposure, hours	Accumulated emergence in per cent of total pupae — 15 days	60 days	130 days	166 days	190 days	335 days	335 days — Per cent mortality	335 days — Per cent alive
Ethylene chlorohydrin	1	.....	2.5	24	0	0	0	0	0	0	100	0
	1a	.....	5.0	24	0	0	0	0	0	0	100	0
	1b	5.0	.....	1	0	0	5	10	10	15	61	24
	1c†	5.0	.....	1	3	4	7	20	21	29	59	12
	1d	10.0	.....	1	1	1	2	11	11	13	66	21
	1e	20.0	.....	1	0	1	3	22	25	35	44	21
Ethylene dichloride	2	.....	2.0	24	0	0	0	0	0	0	100	0
Potassium thiocyanate	3	5.0	.....	1	0	0	2	24	30	37	49	14
	3a	10.0	.....	1	1	2	4	39	56	62	35	3
Ammonium thiocyanate	4	5.0	.....	1	0	0	2	18	23	37	45	18
	4a	10.0	.....	1	0	0	2	9	10	17	71	12
Thiourea	5	5.0	.....	1	1	2	6	25	35	53	20	27
	5a	10.0	.....	1	2	4	4	31	43	56	26	18
Carbon bisulfide	6	.....	1.0	24	0	0	0	0	0	0	100	0
	6a	.....	2.0	24	0	0	0	0	0	0	100	0
Carbon tetrachloride	7	.....	2.5	24	0	0	1	1	1	2	98	0
Hydrocyanic acid	8	.....	2.5	24	0	0	0	0	0	0	100	0
Xylene	9	.....	2.5	24	0	0	0	0	0	2	98	0
Control	10	Distilled water	.....		0	1	3	21	25	38	19	43
	11†	Distilled water	.....	1	4	4	5	17	18	23	64	13
	12	No treatment	.....		1	1	5	17	19	30	28	42

* Two hundred pupae were used in each test. All pupae were 45 days old when treated, unless otherwise indicated.
† Pupae one year old.

SEASONAL HISTORY

Host resistance and accumulated soil-temperature conditions during dormancy apparently exert a profound effect upon seasonal activity of *Rhagoletis completa*. Pertinent facts regarding seasonal history for the five-year period of this study are summarized in figure 57.

Adult Emergence.—Official weather records within the infested area indicate that winter temperatures preceding the 1928 season and temperatures during that season were slightly warmer than normal for this period; however they more nearly approached normality than in any following year during this five-year study. The calculated median of adult emergence (time when 50 per cent of total emergence had occurred) was August 17. The number of days elapsed from the time 50 per cent of the larvae pupated was 321. Accumulated temperature totaled 19,232 day-degrees, and the monthly departure from normal averaged +1.7 degrees. Approximately 70 per cent of the pupae that were formed in 1927 emerged in 1928 and are therefore classed as annual generation. The emergence curve, if smoothed, may be considered a normal frequency polygon, indicating normality of emergence.

In 1929 the median of adult emergence occurred on August 24, and the total period that pupae remained in the soil was 328 days, or 7 days longer than in 1928. The total day-degrees of temperature was 19,286, only 55 degrees more than in 1928, and the monthly departure from normal was –13.8 degrees. Approximately 45 per cent of the 1928 pupae constituted the annual generation. The emergence curve was of the same general shape as that of 1928. In comparing the emergence in 1928 with that of 1929, the data indicate that in 1929 the median was delayed as a result of temperature conditions during dormancy of the pupae.

In 1930 the median of adult emergence occurred on July 29, and the total period that pupae remained in the soil was 306 days, which was 22 days shorter than in 1929, and 15 days shorter than in 1928. The total day-degrees of temperature was 18,313, and the monthly departure from normal was +3.6 degrees. Approximately 90 per cent of the 1929 pupae constituted the annual generation. The emergence curve in 1930 is altered in shape materially from that of previous seasons. The multimodal effect was probably the result of temperature conditions during dormancy. If time of emergence is assumed to be regulated solely by accumulated soil temperature, other factors being equal, then these data would indicate that a slight increment of temperature greatly accelerates emergence.
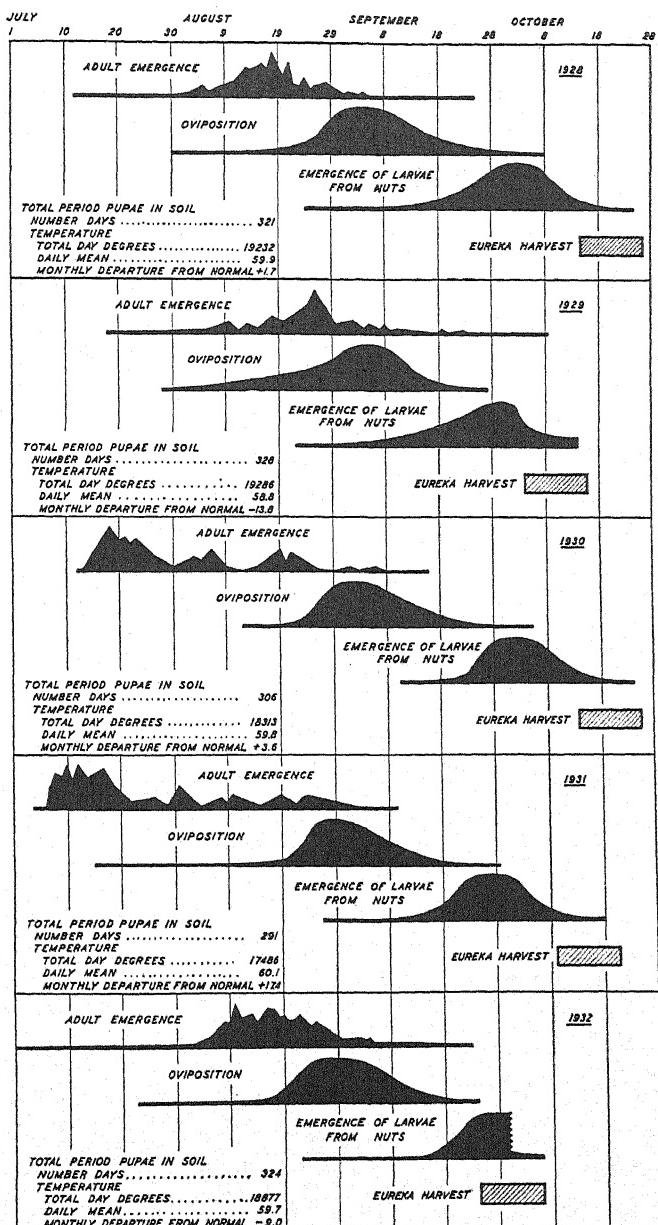
Fig. 57. Seasonal history of *Rhagoletis completa* in the Chino-Pomona area (1928–1932).

In 1931 the median of adult emergence was reached on July 18, and the total period that pupae remained in the soil was 291 days. Thus emergence was 11 days in advance of the previous earliest season, and 25 days in advance of the latest season. The total day-degrees of temperature was 17,486; and the monthly departure from normal was +17.4 degrees. Approximately 83 per cent of the 1930 pupae constituted the annual generation. The shape of the emergence curve probably indicates the operation of abnormal conditions.

At the end of the 1931 season, after four years of study, it became a matter of conjecture whether the early emergence in 1930 and 1931 was wholly due to abnormally high seasonal temperatures or whether the insect was becoming synchronized with the milder climate of this state. The climate of the region in which it is indigenous, particularly the winter season, is rigorous, and adults emerge considerably later in the year than in the latest season here. The matter was economically important here; for, should the flies emerge during the middle of June, it is probable that all varieties of walnuts would be susceptible to infestation.

In 1932 the median of adult emergence occurred on August 16, and the total period that pupae remained in the soil was 324 days. Thus emergence was 29 days later than in 1931, and 1 day earlier than in 1928, which was more nearly normal than any other season during these studies. The accumulated day-degrees of temperature was 18,877, or 355 degrees less than the elapsed day-degrees in 1928, and the monthly departure from normal was −9.0 degrees. Approximately 67 per cent of the 1931 pupae constituted the annual generation. The shape of the 1932 emergence curve approached normality, again resembling that of the 1928 and 1929 seasons.

In consideration of the available information, it appears that an unknown combination of temperatures during dormancy is the most important physical factor governing the time of emergence and also the percentage of individuals that constitute the annual generation. The 1928 and 1929 data indicate that under the existing fluctuating temperature conditions prevailing during the period of dormancy, when a certain amount of day-degrees of temperature was accumulated, transformation to adulthood was initiated. This suggests that the transformation to adulthood represents the completion of physiological processes that for the most part are dependent upon a combination of temperature conditions peculiar to insects with this type of diapause. Continuing this line of reasoning further, the 1930 and 1931 data possibly indicate that these physiological processes are consummated in a shorter period of time and with less actual heat units when the temperatures are higher

during the warm phase of the fluctuating temperature cycle. On this hypothesis the 1932 data indicate that the insect actually is becoming acclimatized, for with subnormally low temperatures during dormancy, transformation to adulthood occurred in practically the same number of days as in 1928, and with fewer day-degrees temperature. Should these generalizations be founded on facts, it follows that in normal seasons the median of adult emergence would probably be reached early in August instead of during the middle of August, which was the case during the earlier history of the insect in its new habitat.

Oviposition Period.—Extensive data regarding hardness of the green walnut husk indicate that this physical factor is the most important of all relating to varietal susceptibility as well as time of oviposition in a susceptible variety. It is interesting to note (fig. 57) that the peak of oviposition each year for the five-year period was reached between August 29 and September 5, despite the wide variation in time of emergence. In each season except 1929 the ascending slope of the oviposition curve was relatively steep, indicating, in 1930 and 1931 particularly, that an inhibitory factor lost its effect rather suddenly. To visualize the situation, females were vainly attempting to oviposit and were not successful until the husk softened sufficiently to permit insertion of the ovipositor. The green husks increase in hardness from the time they are formed until the latter part of June, when the peak of hardness is reached. They gradually soften with approaching maturity. The husk apparently reaches a susceptible condition for oviposition on nearly the same calendar date each year. Should this feature of host susceptibility become an established fact, after a few years of further observation, it would greatly reduce the expense incident to control measures, since one properly timed application would probably be satisfactory. Furthermore the time for treatment would probably become a calendar date, thereby ignoring the emergence of flies for practical purposes of treatment.

Emergence of Larvae from Nuts, and Time of Harvest.—It is evident from figure 57 that during seasons when harvest did not begin before October 10, the greater percentage of larvae had emerged prior to harvest. In 1929 the walnuts ripened early with the result that many contained immature larvae when harvested. The 1932 harvest was the earliest encountered in these studies. Less than half of the larvae had emerged from the nuts and many failed to mature. However, in most instances the larvae had developed sufficiently to cause the shell of the nut to become stained; therefore the extent of injury and resultant loss was not materially mitigated.

NATURAL ENEMIES

Rhagoletis completa is remarkably free from important natural enemies. The total effect of the activities of those species that do attack this insect apparently does not increase environmental resistance sufficiently to constitute an important factor in the economy of the species.

Fungi.—Several species of pathogenic fungi that bring about a mortality among adults under laboratory conditions have been recorded. These species are: *Metarrhizium anisopliae, Botrytis bassiana, Entomophthora* sp., and *Cladosporium* sp. (determined by Charles). These fungi have been found only on flies confined in the battery-jar cages. Occasionally all larvae inhabiting a walnut are found dead in the husk tissue, apparently as a result of a fungal or bacterial pathogen. *Fusarium* sp. has been cultured from these larvae in several instances.

Arachnoid Species.—The mite *Pediculoides ventricosus* New. (determined by Ewing) has been occasionally observed feeding upon the eggs of the walnut husk fly, inside the egg cavity. In all instances a single mite was present and had consumed more than half of the eggs. The mite had entered the cavity through the small puncture made by the female in ovipositing but was so engorged when observed that it occupied nearly one-half of the cavity. Apparently they do not become abundant enough to reduce the numbers of their host materially.

The following spiders, determined by Banks, have been observed preying upon either larvae or adults of the walnut husk fly: *Agelena pacifica* Bks., *Epeira prompta* Htz., *Epeira* sp., *Aysha decepta* Bks., *Zelotes* sp., *Icius vitis* Ckll., *Tetragnatha laboriosa* Htz., *Psilochorus apicalis* Bks., *Pardosa sternalis* Thor., *Ebo* sp., and another *Ebo* sp. which is probably undescribed. While immature and adult spiders utilize appreciable numbers of larvae and adults for food, they are of minor importance in the control of the fly.

Hexapod Species.—Nymphs and adults of the anthocorid, *Triphleps insidiosus* (Say) (determined by Van Duzee), have been commonly observed feeding upon the eggs of the fly. They insert their beaks into the egg cavity through the hole in the husk made for oviposition. In the instances observed, dissection of the cavity immediately after their feeding showed that less than one-half of the eggs present had collapsed. Detailed studies previously reported indicated that approximately 12 per cent of egg mortality at that time could be attributed to the work of natural enemies, probably by this bug and the *Pediculoides* mite. The reduviid, *Zelus renardi* Koln., has commonly been noted with an adult fly impaled on its beak.

The chrysopid, *Chrysopa californica* Coq., and particularly the larvae as they approach maturity, prey upon adults of the walnut husk fly whenever they succeed in capturing them.

Three species of ants (determined by Smith) have been observed preying upon either larvae or adults of the fly. A large grayish species, *Formica cinerea* subsp. *pilicornis* Emery, captures adults as they emerge from the soil and also on the foliage, particularly at night when the flies do not move about to any appreciable extent. This ant also has been observed while transporting larvae to its nests. The tiny thief ant, *Solenopsis molesta* var. *validiuscula* Emery frequently raided the cages in the field laboratory until isolation in water was practiced. They destroyed adults, larvae, and pupae. The tiny black ant, *Monomorium minimum* Buck., destroyed appreciable numbers of larvae in the husks of black walnuts. These nuts usually drop to the ground early in the season before the larvae reach maturity; consequently larval development continues within the walnut lying on the soil.

Throughout the course of these investigations, which involved thousands of larvae and pupae, special efforts have been made to determine the existence of parasites. None has been observed. H. S. Smith conducted a special search for parasites of the larvae in Kansas, the region in which the species is undoubtedly indigenous. He found none; apparently not even the dipterous larval parasites of general feeding habits attack this species. However, two general-feeding parasites of dipterous pupae, the chalcid *Spalangia rugosicollis* Ash. and the proctotrupid, *Galesus* sp., very near *atricornis* Ash. (determined by Gahan), were reared from *Rhagoletis completa* pupae at Manhattan, Kansas, in 1931. They appear to be of negligible importance.

Through the coöperation of the United States Bureau of Entomology, Smith introduced into California from Hawaii the opiine larval parasites *Opius humilis* Silv. and *Diachasma tryoni* Cam. Both species were reared under laboratory conditions from *Rhagoletis completa*. Field colonizations were made in 1931 and 1932 and *O. humilis* was recovered in 1932; however to date *D. tryoni* has not been taken in the field.

Other Animals.—Fowls in general, particularly chickens, and birds eat the larvae and pupae; but their activities are limited and are unimportant.

SCAVENGER SPECIES INHABITING DECAYING WALNUT HUSKS

More than 30 species of insects with scavenger habits have been found associated with or immediately following *Rhagoletis completa* larvae in the decaying walnut husk. Many of those recorded are dipterous larvae from which adults have been reared. Only a few of the more common ones have been determined; they are: *Euxesta putricola* Cole (determined by Cole), *Lonchaea occidentalis* Mall., *Muscina assimilis* Fall, *Fannia canicularis* (Linn.) (all determined by Aldrich), and *Drosophila* spp. The first two species are usually present in all decaying walnut husks, though in certain seasons one species may become more abundant than the other. There is a continuous breeding of these scavenger flies. Late in the season, after walnut husk fly larvae have emerged, larvae of various sizes and comprising several species, are usually abundant.

Several species of beetles have been recorded, though *Carpophilus hemipterus* (Linn.) is most common. A staphylinid species, predacious upon the dipterous scavenger larvae present, usually became abundant late in the season.

The scavenger species increase the husk injury caused by the walnut husk fly; however, their presence seldom appreciably increases the economic loss to the producer.

CONTROL STUDIES

Preliminary life-history data at the beginning of these studies clearly indicated that the adult stage is the most vulnerable to mechanical control practices. The major portion of this phase of the study has therefore been devoted to determining the relative merits of the more promising available toxic materials for destroying the adult and also determining their effect on the walnut tree.

Acid lead arsenate has been used since 1912 in controlling the apple maggot, *Rhagoletis pomonella* (Walsh) and the cherry fruit flies, *R. fausta* (O.S.) and *R. cingulata* (Loew). It was also used against the Mediterranean fruit fly, *Ceratitis capitata* Wied. in the eradication campaign in Florida. But since walnut foliage is very susceptible to injury from acid lead arsenate, this material was eliminated from consideration for field trials. The basic type of lead arsenate does not cause injury and is used extensively on walnuts for the control of the codling moth, *Carpocapsa pomonella*. At the beginning of this study, basic lead arsenate was the only known promising insecticide used as a stomach

poison that could be applied to walnut foliage with safety. However, there was uncertainty as to the expected efficacy of it in comparison with acid lead arsenate since it contains approximately 30 per cent less arsenic oxide (AS_2O_5) with generally a reduction of the amount that is water-soluble.

The control studies are treated under two categories, namely, laboratory investigations and field investigations. The laboratory work extended from 1929 to 1932, while the field work began in 1928 and extended through 1932. Since these investigations were conducted concurrently with the life history and other biological studies, the combined data of any one year somewhat influenced the procedure of the following year.

LABORATORY TOXICOLOGICAL INVESTIGATIONS

Reliable information concerning the toxic effect of basic lead arsenate and other materials on the walnut husk fly was necessary in order to guide future field trials. Therefore experiments were undertaken to obtain this information. They consisted in testing materials under comparable conditions to determine the relative effectiveness of each as measured by the speed of fatality. Some difficulty was experienced in working out a satisfactory technique, particularly with reference to such factors as suitable food and cages for favorable conditions for the flies, rapid handling of the flies in fairly large numbers without inflicting injury, application of standard amounts of materials to be tested, and maintenance of host material bearing toxic elements in a satisfactory condition to insure significant results. The methods devised and used for conducting these studies served the purpose fairly satisfactorily, though they were not refined to a degree suitable for highly accurate toxicological studies such as the determination of mean lethal concentrations. All tests were conducted in a shaded, screened laboratory located in a walnut grove.

Procedure.—Walnut twigs of the Eureka variety, bearing two nuts each, were used as the unit of each test. An effort was made to secure nuts of uniform size for each series. They were taken from unsprayed trees. The twig was cut at a point 8 or 10 inches back from the nuts and immediately placed in water. Each nut was washed in several changes of tap water, as were the bottles into which they were subsequently placed. With bottles partially filled with water, several inches of the stem were cut off and the remaining portion bearing the nuts was placed in the bottle and allowed to dry in the sun. Then absorbent cotton was tightly packed around the stem to support it in an upright position. The cotton also served to prevent the flies from becoming trapped in the water.

It was necessary to provide the flies with some form of food, since repeated tests earlier had shown that they die very rapidly when confined on walnuts under cage conditions. Therefore a stock solution containing 10 grams of granulated cane sugar to 100 cc of tap water was used as the liquid portion of the mixture to be atomized onto the nuts. In the 1931 tests the amount of sugar used was increased to 20 grams. Most of the inorganic stomach poisons were tested at a concentration of 1 :100. (Concentrations of each material tested are indicated in the charts summarizing results.) Approximately 5 cc of the mixture was atomized onto each unit of two nuts with a De Vilbiss atomizer from a standard distance, the atomizer being shaken continuously during the application. Controls received stock sugar solution only, and in amounts equal to those received by other units of the series.

Where the materials were applied as a dust, various concentrations were used ranging from 20 to 90 per cent, by weight, in the regular tests, to 100 per cent in certain special tests. The remaining portion of the dust mixture consisted of from 10 to 50 per cent powdered cane sugar plus the necessary percentage of diluent to bring the mixture up to 100 per cent. Hydrated lime was used as a diluent for the arsenical and copper compounds; while talc, diatomaceous earth, and bentonite were used for the fluorines and other materials. Application was made with a De Vilbiss dust applicator. Approximately 1 gram of the dust mixture was applied to the unit of two nuts. Some controls in the dusted series received an application of powdered cane sugar, while those used to determine whether or not the diluent material effected mortality were treated with a mixture of the particular material and 10 or 20 per cent powdered cane sugar.

The walnuts were placed in the inverted battery-jar cage, one unit to each cage (fig. 58) immediately after treatment in the dust tests, and as soon as thoroughly dry in the liquid tests. However, in certain tests involving the employment of nicotine or other volatile materials, 16-mesh screen cages were used to obviate a fumigating effect. These cages were similar in size and shape to the battery-jar cages and were handled in a like manner. Flies were confined in the cages to feed upon the treated walnuts. Controls were maintained for each test variation.

In 1931 tests were conducted to determine the effect of certain materials applied as dusts directly onto the bodies of the flies. No dilution of materials was made in these instances. The flies used in each test were placed in a large test tube, 2 inches in diameter. The nozzle of the De Vilbiss dust applicator was inserted through the cotton plug stoppering the tube and a uniform amount of material was blown into the tube. The amount was sufficient to fill the tube with a cloud of dust particles. In

this manner flies occupying the tube were contacted by the dust particles which adhered to their bodies in a fairly uniform manner. As soon as the dust cloud settled, the flies were removed and placed in the inverted battery-jar cage containing a cluster of two nuts bearing a coating of stock sugar solution. Two sets of controls were included in each series of these tests: in one powdered cane sugar was dusted onto the insects and in the other the regular operation was simulated except that
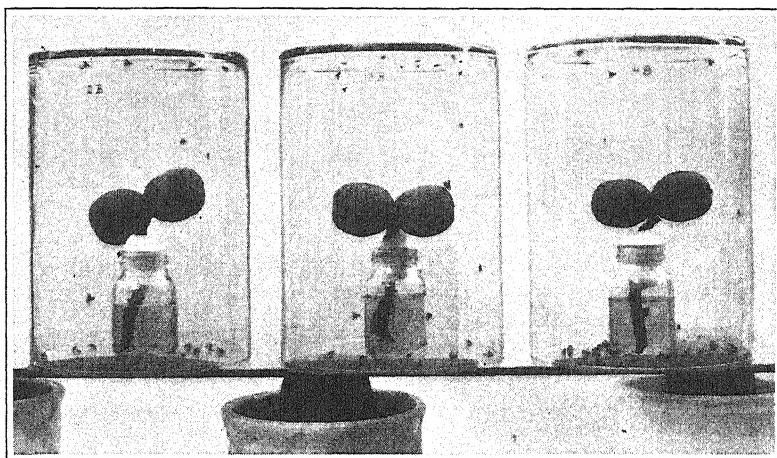
Fig. 58. Inverted battery-jar cage and typical set-up used in toxicity studies.

air void of dust materials was blown into the tube. These tests will be referred to in future discussion as "contact" series.

The flies were handled in the manner previously described. In tests within a series they were usually the same age, though in some cases there was a difference of 1 day. In no instance were the flies over 4 days old when the experiment was set up. Approximately equal numbers of each sex were used. Generally from 20 to 50 flies were used in each test, according to the number available at the time the series was started. However, the number was practically constant within each unit of a series. Flies used were kept in stock cages for 1 or 2 days after they had emerged from the soil, and those injured during collection from the emergence cages were not included in the toxicity series. Since all tests within a series were set up on the same day, and under comparable conditions, the daily mortality rate was regarded as an index of the relative merits of each material tested. The number of dead flies in each test within the regular series was recorded three times daily, while those in the contact series were recorded at 2-hour intervals.

Interpretation of Data.—From a study of literature pertaining to insect toxicology it is apparent that a satisfactory comparison of the toxicity of insecticides at given concentrations can be made when the average time required for 50 per cent mortality is used as a standard. Relative to this matter, Fisher, according to Tattersfield and Morris,[37] states:

> The relation between concentration and probability of death could theoretically be determined by experiment by exposing a large number of insects to the action of the insecticide at each concentration. However, the number of insects required increases enormously if we wish to explore in this manner the region in which the probability of death is high. If as many as 99 per cent of the insects were killed, the accuracy of the comparison between any two insecticides would depend upon the comparatively few insects which survived; and to compare them with any accuracy many thousands of insects would have to be used. The same difficulty arises in the comparatively unimportant case when the deaths are few. For a given number of insects the most accurate comparison can be made when the concentrations are such that about 50 per cent perish. The region between 25 per cent and 75 per cent can be fairly easily explored. It is for this reason that the preliminary examination of chemical substances should be made by comparison of the concentrations required to give a mortality of 50 per cent. When the equivalent at this point is established it would be most valuable to ascertain if the same relative concentrations are equivalent over the range 25–75 per cent. Only in this way does it seem possible to infer a general equivalence of insecticidal properties. The direct comparison of mortality when the probability of survival is very small would seem to be beyond the scope of accurate laboratory investigation.

Bar diagrams are employed in figures 59, 62, 65, and 69 to present the data obtained from these studies, using the average time required for 50 per cent mortality as the standard of comparison.

Figures 60, 61, 63, 64, 66, 67, and 68 show in more detail the relative effectiveness of the materials tested, the standard of comparison being the average time required for mortality of from 25 to 75 per cent. Plotted on semilog paper, these points fall fairly well along a straight line. Throughout this range the data indicate the regular sigmoid toxicity curve relation.[38] The curves have not been mathematically fitted, since the conclusions to be drawn from the data do not appear to warrant such detailed treatment.

Experiments in 1929.—Data obtained from laboratory studies of the effectiveness of the various materials tested in 1929 are presented in figures 59, 60, and 61.

The arsenicals tested were effective in causing mortality of the flies. These tests show that the speed of toxic action bears a direct relation to the arsenic content and degree of solubility in water. Basic lead arsenate applied as dust was more rapid in its action than when applied as spray

MATERIAL AND CONCENTRATION	1929	HOW APPLIED	NUMBER TESTS	TOTAL NUMBER FLIES	MEAN-HOURS REQUIRED FOR 50 PERCENT MORTALITY
CONTROL	10% SUCROSE	SPRAY	4	114	
	POWD. SUCROSE	DUST	4	111	
CALCIUM FLUORIDE	1:100	SPRAY	3	70	
CA. FLUOSILICATE COMPOUND	1:100	SPRAY	3	59	
BASIC LEAD ARSENATE	1:100	SPRAY	3	73	
(LIME AS DILUENT)	20%	DUST	3	74	
ACID LEAD ARSENATE	1:100	SPRAY	3	51	
COPPER SULFATE	1:100	SPRAY	3	132	
BARIUM FLUORIDE	20%	DUST	3	58	
BARIUM FLUOSILICATE	1:100	SPRAY	2	53	
(TALC AS DILUENT)	20%	DUST	2	59	
SODIUM FLUORIDE	1:100	SPRAY	1	20	
(TALC AS DILUENT)	20%	DUST	2	39	
MG. FLUOSILICATE	1:100	SPRAY	2	37	
SODIUM FLUOSILICATE	1:100	SPRAY	3	72	
(TALC AS DILUENT)	20%	DUST	4	96	
CALCIUM ARSENATE	1:100	SPRAY	2	45	
SODIUM ARSENITE	1:100	SPRAY	2	40	
NICOTINE SULFATE	5:100	SPRAY	2	71	
TOTALS			51	1274	

Fig. 59. Results of toxicity studies in 1929, showing number of hours required to bring about 50 per cent mortality of *Rhagoletis completa.*
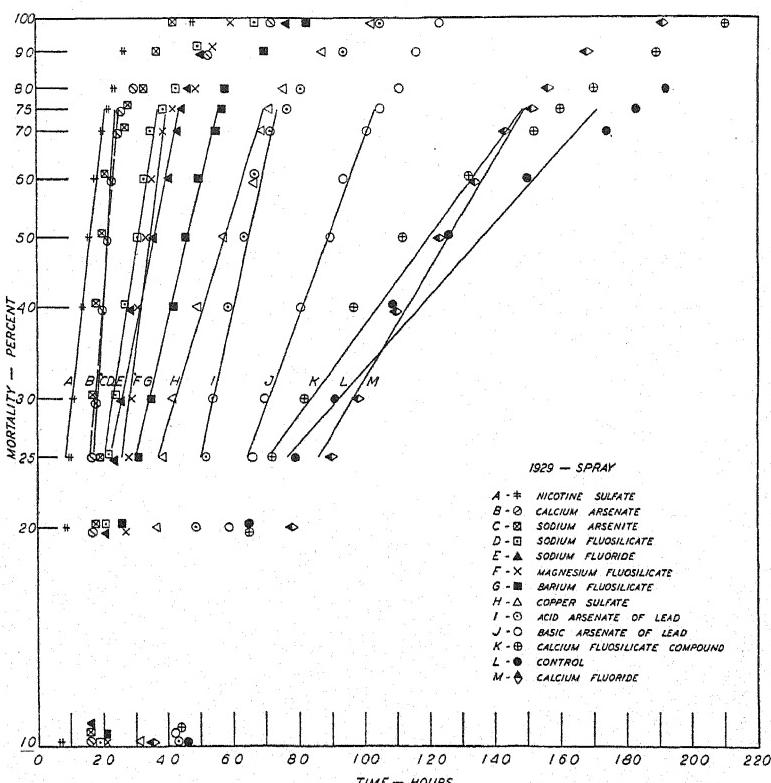
Fig. 60. Relative effectiveness of various materials applied as spray in 1929. Standard of comparison is the mean mortality period from 25 to 75 per cent.

and even more rapid than the acid lead arsenate applied as spray. Since comparable amounts of these materials, by weight, were applied, perhaps the dust particles are more readily ingested by the flies. Furthermore previous work had established the fact that more material was retained by the surface of the walnut in dust tests than in spray tests, and
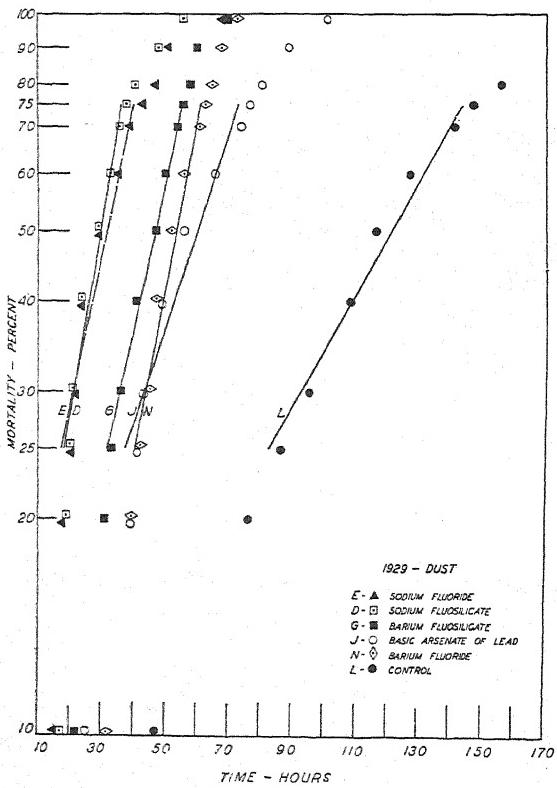
Fig. 61. Relative effectiveness of various materials applied as dust in 1929. Standard of comparison is the mean mortality period from 25 to 75 per cent.

the amount retained, both of basic and acid lead arsenates, adheres to plant surfaces more firmly when applied as spray than when dusted.

All fluorines tested, except those combined with calcium, killed the flies fairly rapidly. The speed of toxic action is correlated with the solubility of the material in water, the more soluble compounds being more rapid in their lethal action. Calcium fluoride and calcium fluosilicate compound were not effective, which fact may possibly be corroboratory evidence regarding the theory of action of the fluorines in bringing

about insect mortality:[26] that they kill by virtue of the affinity of fluorine for calcium, the fluorine precipitating calcium in the body tissues, thereby interfering with permeability. On this basis a possible conclusion is that the affinity of the fluorine in the calcium combinations tested is already satisfied, partially or wholly, thereby rendering them ineffective. However, the solubility of the calcium combinations tested is very low, which fact alone may account for the low insecticidal efficiency.
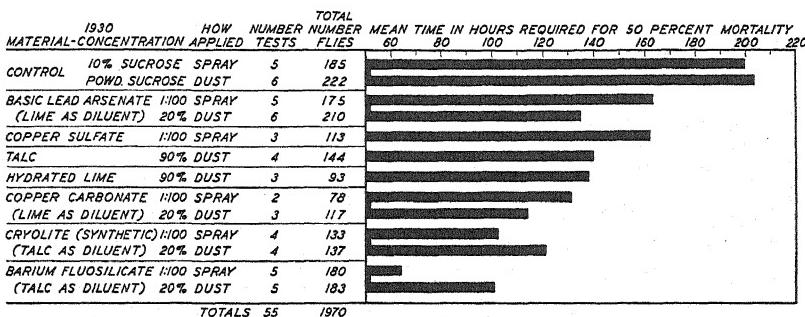
Fig. 62. Results of toxicity studies in 1930, showing number of hours required to bring about 50 per cent mortality of *Rhagoletis completa.*

Nicotine sulfate was employed as a stomach poison. The sugar of the stock food solution apparently reduced the volatilization of the nicotine. The action of nicotine in these tests was the most rapid of the materials tested.

Copper sulfate was more rapid in its action than acid lead arsenate. The nature of the lethal action of copper is not understood; it may kill directly by poisoning, or indirectly by destroying the intestinal microorganisms.

Tree-tolerance tests in 1929 eliminated acid lead arsenate, calcium arsenate, sodium arsenite, sodium fluoride, sodium fluosilicate, and magnesium fluosilicate from further trials except for comparative toxicity purposes, since important injury resulted from their use. Calcium fluoride and calcium fluosilicate compound were eliminated because of low insecticidal efficiency. Barium fluoride was not continued since it was less practicable and no more efficacious than barium fluosilicate.

Experiments in 1930.—Data obtained from laboratory studies of the effectiveness of various materials tested in 1930 are presented in figures 62, 63, and 64. The speed of toxic action of certain materials was considerably slower in 1930 than in 1929. This variable longevity factor is likewise evident when a comparison is made of the length of life in the

controls for both seasons. The explanation of these differences is not known. Materials and technique were identical in both seasons. However, the performance of materials used both seasons and the controls show a fairly close ratio of variation, i.e., basic lead arsenate required twice as long to bring about 50 per cent mortality in 1930 as in 1929, and
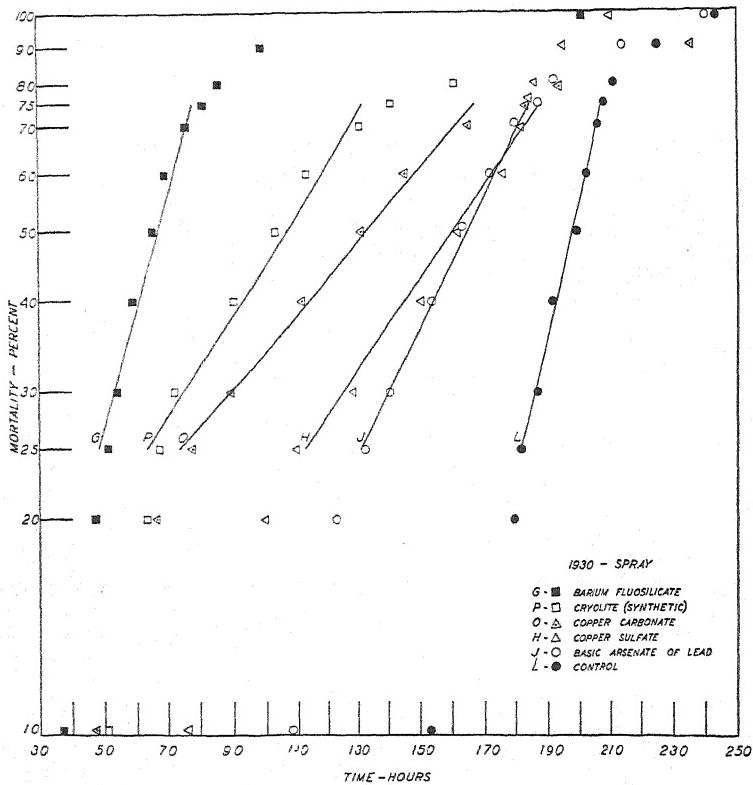
Fig. 63. Relative effectiveness of various materials applied as spray in 1930. Standard of comparison is mean mortality period from 25 to 75 per cent.

controls lived about 1.6 times as long in 1930 as in 1929. Climatic conditions may have been the influencing factor; temperature and humidity control was not undertaken in any of these studies.

Basic lead arsenate, applied as dust, exhibited more rapid action than the spray, as in 1929.

Copper sulfate was not as effective as in 1929, and copper carbonate surpassed the sulfate form in speed of toxic action. Here again the dust was more rapid than the spray in killing the flies. Both materials were eliminated from further trials because of injury to walnut foliage in field trials.

Of the fluorines tested, barium fluosilicate was superior to synthetic cryolite (sodium fluoaluminate). With both materials the spray tests showed a more rapid killing effect than dust tests.

Talc and hydrated lime both exhibited insecticidal action. The mortality rates were closely approximate to each other and also to that of
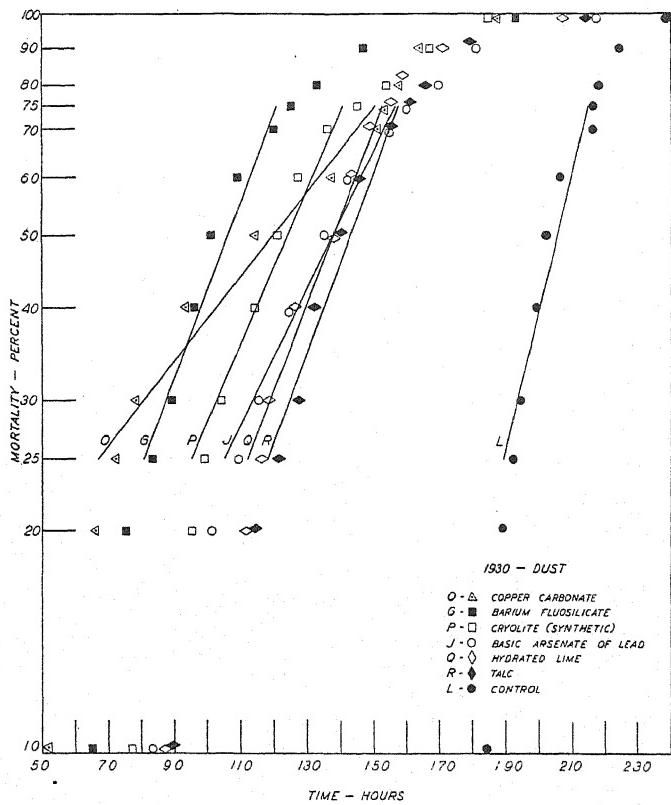
Fig. 64. Relative effectiveness of various materials applied as dust in 1930. Standard of comparison is mean mortality period from 25 to 75 per cent.

basic lead arsenate applied as a dust. The nature of action of talc and hydrated lime is not understood.

Experiments in 1931.—Data obtained from laboratory studies of the effectiveness of various materials tested in 1931 are presented in figures 65, 66, 67, and 68.

The controls in 1931 show that the inverted battery-jar cages afford more favorable conditions for longevity than do the screen cages. Furthermore when the food (sucrose) is supplied as a spray the flies live

1931 MATERIAL AND CONCENTRATION		HOW APPLIED	NUMBER TESTS	TOTAL NUMBER FLIES
CONTROLS	GLASS CAGE – 20% SUCROSE	SPRAY	3	102
	SCREEN CAGE – 20% SUCROSE	SPRAY	3	107
	GLASS CAGE – POWD. SUCROSE	DUST	4	138
	SCREEN CAGE – POWD. SUCROSE	DUST	4	88
	(CONTACT)¹ GLASS CAGE – POWD. SUCROSE	DUST	4	96
	(CONTACT) GLASS CAGE – POWD. SUCROSE	DUST	6	160
DIATOMACEOUS EARTH	80% + 12% WHITE OIL	DUST	3	58
	80%	DUST	3	97
	(CONTACT)¹ 100%	DUST	5	128
TALC	80%	DUST	2	61
	(CONTACT)¹ 100%	DUST	4	102
SULFUR	80%	DUST	2	59
	(CONTACT)¹ 100%	DUST	4	100
BENTONITE	(CONTACT)¹ 100%	DUST	4	102
	80%	DUST	2	56
HYDRATED LIME	80%	DUST	4	57
	(CONTACT)¹ 100%	DUST	4	96
BASIC LEAD ARSENATE (LIME AS DILUENT)	1:100	SPRAY	2	47
	20%	DUST	2	47
	40%	DUST	2	60
CRYOLITE (NATURAL) (TALC AS DILUENT)	1:100	SPRAY	2	49
	20%	DUST	2	42
	40%	DUST	2	59
	(CONTACT)¹ 100%	DUST	4	100
BARIUM FLUOSILICATE (TALC AS DILUENT)	20% + 12% FISH OIL	SPRAY	2	62
	20%	DUST	2	56
	40%	DUST	2	55
	(CONTACT)¹ 100%	DUST	2	63
	(CONTACT)¹ 100%	DUST	4	103
CRYOLITE (SYNTHETIC) (TALC AS DILUENT)	1:100	SPRAY	2	59
	20%	DUST	2	67
	40%	DUST	2	60
	(CONTACT)¹ 100%	DUST	4	100
NICOTINE SULPHATE – TANNIC ACID	1 – 3:500²	SPRAY	2	67
NICOTINE SULPHATE – BUTANOL – WHITE OIL	1 – 1 – 15:500²	SPRAY	2	71
NICOTINE SULPHATE – SUCROSE	1 – 50:500²	SPRAY	2	64
NICOTINE 2% IN BENTONITE²	80%	DUST	2	54
NICOTINE 2% IN DIATOM. EARTH²	80%	DUST	2	66
NICOTINE 2% (GROUND TOBACCO)²	80%	DUST	2	70
		TOTALS	106	3028

NOTE
¹ MATERIAL APPLIED DIRECTLY ON BODIES OF INSECTS
² SCREEN CAGES USED

Fig. 65. Results of toxicity studies in 1931, showing number of hours required to bring about 50 per cent mortality of *Rhagoletis completa.*

longer in screen cages than when powdered sugar is dusted onto the
nuts. Powdered sucrose applied directly to the bodies of the flies had no
deleterious effect upon them.
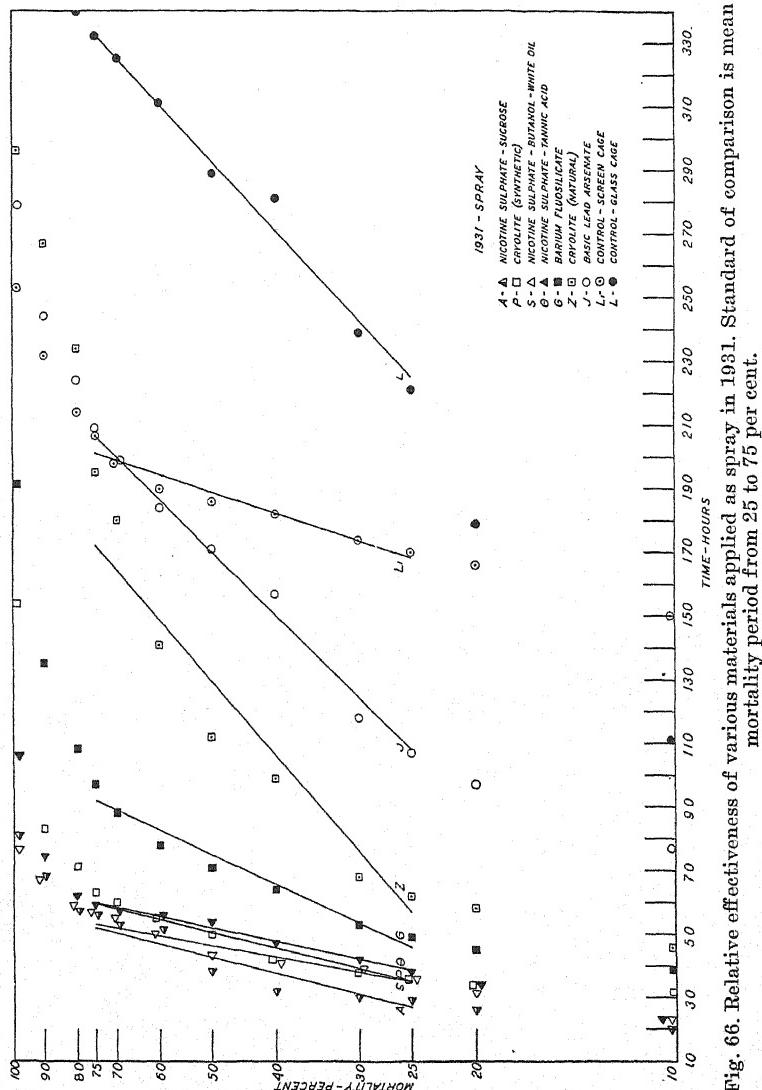

Fig. 66. Relative effectiveness of various materials applied as spray in 1931. Standard of comparison is mean mortality period from 25 to 75 per cent.

In the contact series the mortality caused by the contact of the ma-
terials with the bodies was very probably an indirect effect, since they
undoubtedly ingested relatively large amounts of the material as a re-
sult of their "cleaning-up" habits. For several hours after treatment
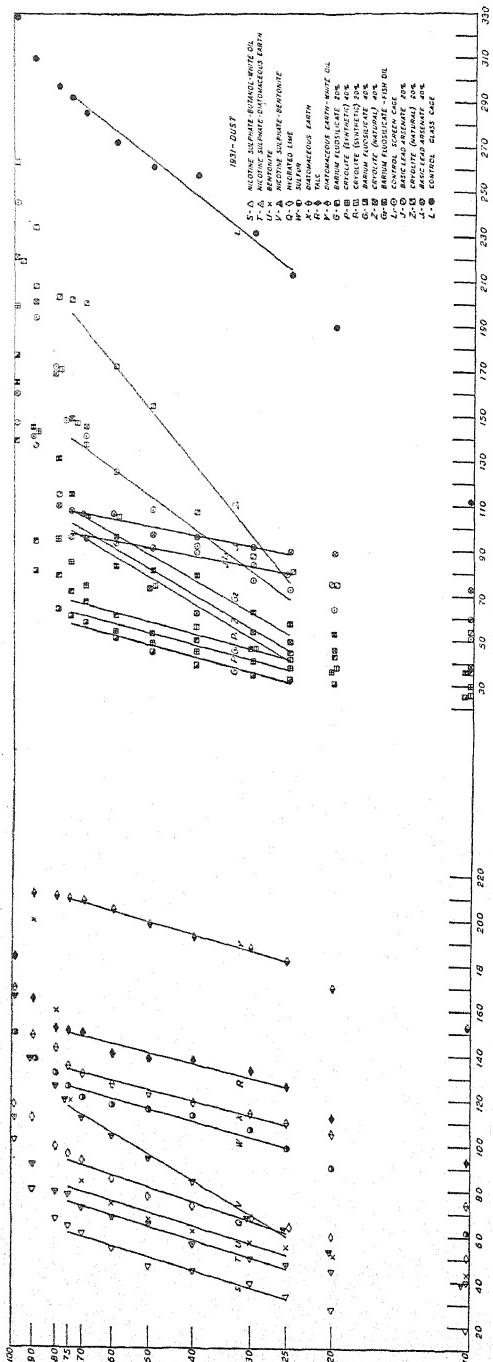
Fig. 67. Relative effectiveness of various materials applied as dust in 1931. Standard of comparison is mean mortality period from 25 to 75 per cent.

they were observed to be constantly removing the dust from their bodies with their legs, the fore pair usually being cleaned with the mouth parts.

Of all materials used, diatomaceous earth was the most rapid in its lethal action when applied directly onto the bodies of the flies. It pro-
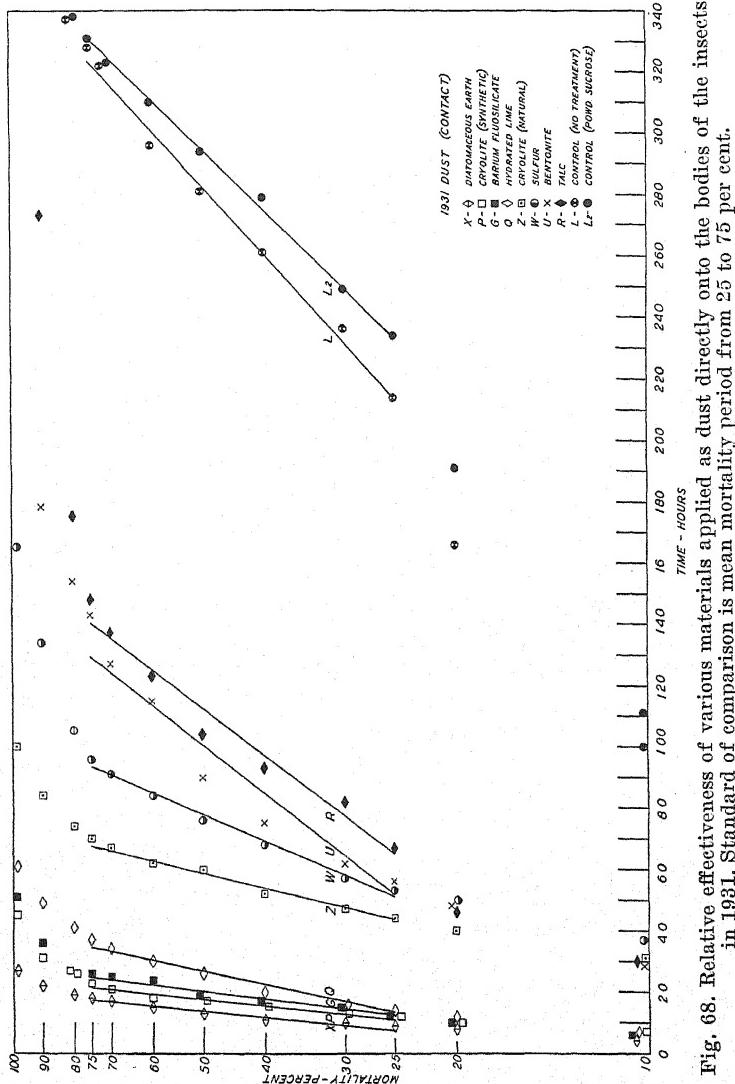
Fig. 68. Relative effectiveness of various materials applied as dust directly onto the bodies of the insects in 1931. Standard of comparison is mean mortality period from 25 to 75 per cent.

duced mortality much more slowly when flies fed upon it normally, and still more slowly when mineral oil was incorporated therein. Oil causes the earth particles to adhere more firmly to the surface of the nut,

thereby apparently rendering it more difficult for the flies to pick up in feeding. Talc, sulfur, bentonite, and hydrated lime were also more rapid in their action when applied directly onto the bodies of the flies than when flies fed normally upon them. Apparently larger amounts of material are ingested during the removal of the material from their bodies than through normal feeding.

All diluent materials used, in dusts applied to the nuts, that is, diatomaceous earth, talc, sulfur, bentonite, and hydrated lime, demonstrated lethal action upon the flies, the latter being generally more rapid than the others. The nature of this action is unknown; however, the following theories are suggested, although they are highly problematical. Diatomaceous earth and talc may seriously abrade the intima and epithelium, and possibly other tissues of the alimentary canal. Furthermore, they may produce a diarrheal condition as a result of the cathartic action of magnesium, the content of which is relatively high. Sulfur may produce a highly acid condition in the digestive system, thereby causing deleterious metabolic disorders. Bentonite is colloidal in nature and apparently withdraws moisture from the intestinal walls; however, instead of the usual flushing action, there is a clogging of the rectum as a result of increased volume due to water adsorption. Therefore, death may ensue as a result of failure to eliminate excrement. Hydrated lime may produce a somewhat hardened layer on the inner lining of the intestine, resulting in a failure to digest and assimilate food, thereby causing mortality through starvation, which may likewise be an important factor in mortality produced by diatomaceous earth, talc, and sulfur.

Considering the known toxicity of nicotine, the nicotine compound and mixtures tested were relatively slow in their action as stomach poisons. The differences evidenced in speed of action of the various mixtures possibly indicate the degree to which the nicotine is bound within or by the respective compounds and mixtures.

Basic lead arsenate exhibited relatively slow action; and spray tests were slower in producing mortality than were dust tests. The performance of this material in 1931 was in accord with the 1929 and 1930 tests. The 40 per cent dust mixture was somewhat slower in action than the 20 per cent mixture. Considering the speed of action of hydrated lime, it is possible that the increased amount of this diluent material in the 20 per cent lead arsenate mixture was responsible for the observed difference in speed of action over the 40 per cent lead arsenate mixture.

Barium fluosilicate and synthetic cryolite (sodium fluoaluminate) on the whole exhibited similar speeds of toxic action. Fish oil incorporated in the barium fluosilicate mixture appreciably retarded the effect of the latter. The explanation of this fact is presumably that sug-

gested previously for the diatomaceous earth and mineral oil mixture. Observation showed that the fish oil was not repellent to the flies. In the barium fluosilicate dust tests, the 40 per cent mixture was slower in its action than the 20 per cent mixture, which fact is not readily explained. In speed of action, natural cryolite was considerably inferior to the synthetic material in these tests. Similar results were obtained by Ripley and Hepburn[34] in toxicity studies dealing with the Natal fruit fly, *Pterandus rosae* (Ksh.). The two materials are identical in composition and the ones used in these tests were similar in physical properties. Pos-
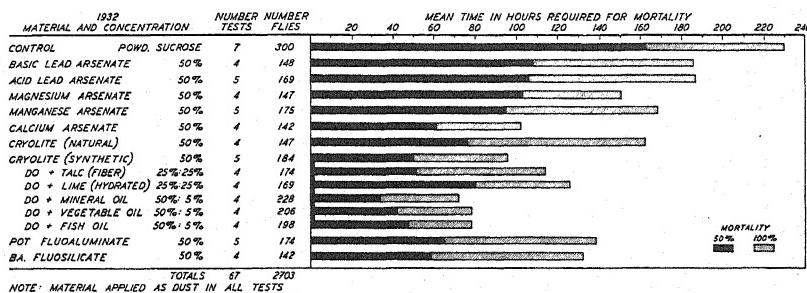
Fig. 69. Results of toxicity studies in 1932, showing number of hours required to bring about 50 per cent and 100 per cent mortality of *Rhagoletis completa*.

sibly the synthetic material is more effective because the bond between the elements of the molecule is not as great as in the natural product, thereby liberating the fluorine more readily.

Experiments in 1932.—Data obtained from laboratory studies of the effectiveness of various materials tested in 1932 are presented in figure 69.

The longevity of flies in the controls was sufficient to attribute significance to the indicated data from tests involving the use of toxic material.

Magnesium arsenate was employed in a field control plot in 1932, while manganese arsenate received consideration in tree-tolerance tests. It was therefore important to determine the relative effectiveness of these two materials in comparison with other arsenicals and fluorine compounds. The data indicate insignificant differences in the speed of action of basic lead arsenate and acid lead arsenate, with slightly increased efficacy for manganese and magnesium arsenate, and greatest rapidity of action for calcium arsenate.

Natural cryolite was more effective than any of the arsenicals tested with the exception of calcium arsenate. However, it was slower in action than any of the other fluorine compounds tested.

Synthetic cryolite exhibited greatest speed of toxic action of all materials tested. Regarding the effect on speed of action when a calcium diluent is used, the data indicate appreciable inhibition. An inert diluent such as talc apparently exerts no effect upon toxic action. The 1931 studies showed that the incorporation of 12 per cent mineral oil or fish oil in dust mixtures for adhesive purposes materially reduced the rate of mortality. Further information regarding this matter was desirable, therefore tests were conducted involving the use of three types of oil. Preliminary studies of mineral oil, vegetable oil, and fish oil as adhesives had shown that satisfactory results were obtained when a 5 per cent concentration of any one was employed. These toxicity studies indicate increased speed of action in the tests where mineral oil and vegetable oil were employed. The differences shown appear to be significant, particularly in the mineral-oil test; however, for the purpose of this investigation the most important consideration is that none of the oils used inhibited toxic action.

Barium fluosilicate and potassium fluoaluminate are highly efficacious materials. The physical properties, particularly the bulkiness of the latter, are superior to other fluorine compounds tested.

Ingestion Studies.—In control studies dealing with insects possessing mouth parts of the sponging and sucking type, the nature of lethal action of certain solid materials with low solubility in water is not definitely understood. Researchers on the *Rhagoletis pomonella* problem have opined that lead arsenate is not ingested by the flies, since the particles are "strained out" of imbibed liquid by the pseudotracheae or other structures on the labella. Thus, in order for the material to cause death, it would be necessary for the flies to ingest appreciable quantities of arsenic in solution. Considering the water-solubility of commercial lead arsenate, it seems improbable that lethal amounts could be obtained in this manner. However, quantities of the material may be readily dissolved in the saliva which the flies emit in normal feeding and which is subsequently imbibed.

In feeding studies on nonbiting flies, Graham-Smith[18] found that the blow fly (*Calliphora erythrocephala* Mg.) readily ingests all particles measuring up to 0.006 mm in diameter, and that particles measuring up to 0.02 mm in diameter are drawn into the pseudotrachea when two opposite interbifid grooves are made to communicate with each other. Furthermore, relatively large objects apparently pass directly into the mouth when the prestomal cavity is open during prolonged sucking efforts made by the flies. Thus these studies show that the structure of this type of mouth part permits ingestion of solid matter. The mouth parts of the blow fly are somewhat similar in structure and size

to those of the walnut husk fly. For enlightenment on this matter, an effort was made to determine whether or not flies actually ingested the solid particles of lead arsenate. In preliminary tests lead arsenate was colored with aniline blue, methyl green, orange *G*, and säurefuchsin, all of which are water-soluble stains. Individual tests were conducted in which the dyed material was dusted onto walnuts and flies caged on them. After several days' confinement under such conditions, dissection showed that the gut of most of the flies contained material similar in color to that with which the lead arsenate was originally dyed. Furthermore, excrement of the color used was present on the sides of the battery-jar cage. This indicated that the flies had ingested the stained particulate matter; however, definite evidence was lacking since only the water-soluble stain itself may have been taken in. Therefore similar tests were conducted in 1929 and 1930, in which non-water-soluble stains were used. Of the many tested, Sudan *III*, which is soluble in alcohol, served the purpose most satisfactorily. A saturated solution of the stain was made and thoroughly mixed with a small amount of the lead arsenate (approximately 20 grams), thus making a paste. This was allowed to dry at laboratory temperature. When completely dry, the material was pulverized and the procedure repeated. After three or four successive mixings with the dissolved stain, the lead arsenate was the color of the stain, scarlet red.

Lead arsenate, barium fluosilicate, cryolite, copper carbonate, hydrated lime, talc, diatomaceous earth, bentonite, sulfur, and ground tobacco leaves were stained in the manner just described. Individual cage tests of each material applied on walnuts as spray and dust were conducted. In these tests the materials were used undiluted, except for the 20 per cent sucrose that was included for food. Controls were maintained in which the walnuts were treated with sucrose only. Fifty flies were used in each test. Approximately 48 hours after the flies were confined on the treated walnuts, lots consisting of 25 flies from each test were removed, stupefied with ether, and dissected. All dissection was performed with the aid of a binocular microscope at a magnification of ×50. In all tests over 98 per cent of the flies dissected had large quantities of stained material present in various portions of the alimentary canal; however, it was not abundant in the hind intestine. Varying amounts were also present on the labella of the proboscis. In the controls, the alimentary canal of all flies dissected was creamy white, except in certain instances where the rectum presented a slightly brownish coloration.

Just prior to stupefaction for dissection, when the vial containing the flies was held so as to permit light to pass through the abdomen, the in-

testine containing stained material was plainly visible in all instances. This was not observed in any of the flies from control cages. In all tests, except where stained bentonite was employed, characteristic reddish excrement was present on the sides of the battery-jar cage. The recta of most of the flies consuming bentonite were greatly distended and upon further dissection proved to be effectively plugged with a somewhat gum-like material. Apparently this colloidal substance had absorbed relatively large amounts of moisture, and greatly increased volume had resulted.

In the diatomaceous-earth tests, microscopic examination of stained contents from various portions of the alimentary canal revealed the presence of diatome shells. Furthermore these minute shells were found in the stained excrement taken from the battery-jar cage of this particular test. These observations demonstrate conclusively that particulate matter is ingested by the flies, while the evidence from the tests involving other materials is considered to be strongly indicative of this fact. Undoubtedly the flies imbibe these undissolved particles in suspension in droplets of dew, or in their own saliva, which is emitted in the process of feeding.

Comments on Toxicological Investigations.—Walnut husk flies are favorable subjects for toxicological studies of this nature, since the death point is readily determined. Furthermore the type of cage used permitted accurate observations on the rate of mortality.

Cage tests of this nature are not actual comparisons of the toxicity of the various materials, as has been pointed out by Campbell.[10] The insects feed freely and consume unknown quantities of the material. However, such tests are deemed satisfactory for comparing the relative effectiveness of materials when such factors as those of biological and environmental nature are comparable. It is recognized that these studies have not been extensive enough or sufficiently well controlled to warrant important toxicological conclusions. A possible partial explanation of erratic results obtained in some instances may be attributed to the time of beginning a series of experiments in relation to the time and the amount of feeding of the flies just prior to being confined on the treated walnuts. Ideally, the flies should not have been permitted to feed at all until placed in the test cages. Thus they would all begin to consume the material at approximately the same time. However, this procedure was not feasible under existing conditions.

These studies have supplied valuable information for field plot trials. Basic lead arsenate has consistently demonstrated its relatively slow action in producing mortality, while barium fluosilicate and synthetic cryolite have been consistently efficacious in this respect.

FIELD INVESTIGATIONS IN 1928

Two experiments were conducted in 1928, using basic lead arsenate. Because of the absence of summer rainfall, the loss of the arsenical from walnut foliage and fruit through weathering during the period of ac-

Fig. 70. Spraying equipment in operation in experimental control plots.

tivity of the fly is probably not very great. Therefore one coverage of this material, applied when the flies begin to emerge, should afford data regarding the efficacy of it in the control of the walnut husk fly.

Plot Experiments I and II.—A plot was sprayed (fig. 70) in each of two groves where the infestation was reported to have been appreciable

TABLE 26

CONTROL EXPERIMENTS IN 1928

Experi-ment No.	Number of trees and variety	Material	Concentration	Date of application	Trees counted	Total nuts counted	Mean per cent infested†	Per cent control
I	(9½ acres)							
A*	43 Eureka	Basic lead arsenate	4 lbs.–100 gals.	Sept. 1	4	831	28	71
B	119 Eureka	Check—no treatment			4	762	96	...
II	(10 acres)							
A*	36 Eureka + 30 Neff	Basic lead arsenate	4 lbs.–100 gals.	Sept. 2	6	1,334	25	65
B	84 Eureka + 70 Neff	Check—no treatment			6	1,480	71	...
Totals	4 plots, 382 walnut trees, 19½ acres				20	4,407	...	...

* One application of material as spray at 20 gallons per tree.

† Data obtained from count of nuts on lower portion of Eureka trees just before harvest (October 14).

in 1927. Each plot was approximately square in shape. The trees were not large and, therefore, 20 gallons of material per tree gave satisfactory coverage. Application was made on August 1 and 2. A few flies emerged in July; however, the daily number did not increase until early in August, reaching the seasonal peak ŏn August 18 (fig. 36).

For evaluation of results counts were made of the percentage of infested nuts on representative trees in the centers of treated plots as well as in the adjoining check plots. The percentage of reduction in infestation or the percentage of control, was calculated after the method used by Porter.[31] $\bar{B}$ represents the mean percentage of infestation in the treated plot and $\bar{A}$ represents the mean percentage of infestation in the untreated plot. Then

$$100 - 100\,\frac{\bar{B}}{\bar{A}} = \text{per cent reduction in infestation or per cent control.}$$

A summary of the data obtained from these experiments is presented in table 26.

The data indicate that basic lead arsenate very materially reduced the degree of infestation. However, with 25 per cent infested nuts in treated plots the degree of control was not satisfactory. The season was apparently very favorable for the walnut husk fly; for heavy infestations developed in groves that were so lightly infested the preceding year as to be unnoticed.

FIELD INVESTIGATIONS IN 1929

The performance of basic lead arsenate in 1928 was sufficiently encouraging to warrant further field trials; and since very little information was available concerning tree reaction from other materials, basic lead arsenate was the only toxic material tested in field control plots in 1929. The so-called "bait sprays" were given a considerable amount of attention. Plots were differentially treated with respect to concentration of materials, amount per tree, number of applications, and the timing of the applications.

It was regrettable that representative controls were not feasible during this season. Authorities were seriously considering an eradication campaign which prohibited the maintenance of any sizable untreated area. The "check" indicated in the several experiments was in all instances a border row contiguous to treated plots. Later studies have shown that such an arrangement does not yield truly significant results. Therefore, in attempting to evaluate the efficacy of the various treatments certain supplemental facts should be considered. The fly population within the experimental groves was sufficiently large to produce

serious damage in all instances had not lethal or other prohibitive factors been in operation. Reliable information regarding fly population was available from two sources. Emergence cages were in operation throughout the season within the respective groves. Furthermore sweetened material sprayed onto the tips of branches of representative trees served to congregate flies during their early-morning feeding activities. In this manner it was possible to note the relative abundance of flies within a grove.

Even though seasonal emergence records show that only approximately 45 per cent of the flies emerged, they were present in relatively large numbers. It is questionable whether or not the varieties infested in previous years were in a susceptible condition during the activities of the flies in 1929. It has been pointed out in the host studies that husk hardness appears to be the most important factor governing oviposition, and that this feature is variable with the same varieties in different groves. However, in several small untreated Eureka groves within the area, fairly heavy infestations developed. This information may or may not be significant in view of the lack of detailed data regarding such features as the ratio of nut population to female population and percentage of infestation.

In order to determine the degree of infestation in the various plots, fairly extensive counts of the nuts on the trees in the centers of the plots were made just prior to harvest. These counts included nuts distributed over all portions of the tree. Nuts were considered infested when eggs had been deposited in the husks.

Harvest was abnormally early. Therefore, an appreciable percentage of those nuts classified as "infested" in field counts failed to exhibit evidence of injury when harvested. Percentage of infestation does not necessarily signify percentage of injury; however, the former is the important consideration in evaluating the merits of the various materials tested.

Adult emergence was somewhat later than in 1928. A few flies emerged during the latter part of July and early August, with the seasonal peak being reached on August 26 (fig. 37, p. 409).

Experimental plots comprised approximately 70 acres and included practically all of the moderate to heavily infested properties in the area in 1928. A summary of these experiments is given in table 27.

Plot Experiment I.—Infested nuts were not found in any of the plots until the latter part of August. Flies were commonly observed in all parts of the grove throughout the entire season. The check trees were about one-half the size of the trees in the treated plots and were in an outer border row.

TABLE 27
CONTROL EXPERIMENTS IN 1929

Experiment	Number of trees and variety	Material	Concentration (per 100 gals. for sprays and per 100 lbs. for dusts)	Application					Estimated per cent infested 1928	Results			
				Method	Am't per tree, gals. for sprays and lbs. for dusts	Number	Date	Foliage injury "burn"		Trees counted	Total nuts counted	Mean* per cent infested	Per cent control*
I (10 acres)													
A	77 Eureka	Basic lead arsenate	4 lbs.	Spray	20	3	7/20, 8/15, 9/4	None	70-90	6	2,262	0.4	94
B	77 Eureka	{Basic lead arsenate / Hydrated lime}	25 lbs. / 75 lbs.	Dust	1½	3	7/20, 8/15, 9/4	None	70-90	6	2,290	0.7	89
C	17 Eureka	Check, no treatment	....	....		...		....	70-90	6	1,986	6.4	...
II (5 acres)													
A	34 Eureka	Basic lead arsenate	4 lbs.	Spray	20	3	7/31, 8/16, 9/4	None	40-60	4	1,283	1.1	93
B	34 Eureka	Basic lead arsenate	100 lbs.	Dust	1	3	7/31, 8/16, 9/4	None	40-60	4	1,309	5.4	66
C	17 Eureka	Check, no treatment	....	....		...		....	40-60	4	904	15.9	...
III (10 acres)													
A	30 Eureka	Basic lead arsenate	4 lbs.	Spray	20	3	7/31, 8/15, 9/5	None	40-60	6	1,084	1.0	77
B	30 Eureka	Basic lead arsenate	4 lbs.	Spray	10	3	7/31, 8/15, 9/5	None	40-60	6	1,190	0.5	88
C	30 Eureka	Basic lead arsenate	4 lbs.	Spray	20	2	8/6, 8/25	None	40-60	6	1,071	0.1	98
D	30 Eureka	Basic lead arsenate	4 lbs.	Spray	10	2	8/6, 8/25	None	40-60	6	1,105	0.3	93
E	17 Eureka	Check, no treatment	....	....		...		....	40-60	6	1,013	4.4	...
IV (5 acres)													
	85 Eureka + 157 peach†	Basic lead arsenate	4 lbs.	Spray	15	3	8/2, 8/16, 9/4	None	20-40	6	1,694	30.1	...
V (4 acres)													
A	34 Eureka	Basic lead arsenate	4 lbs.	Spray	20	3	8/1, 8/19, 9/7	None	10-20	5	1,062	2.4	46‡
B	34 Eureka	{Basic lead arsenate / Corn syrup "Karo"}	4 lbs. / 3 gals.	Spray	20	3	8/1, 8/19, 9/7	Slight	10-20	5	1,051	4.4	...
VI (5½ acres)													
A	43 Eureka	{Basic lead arsenate}	4 lbs. / 6 lbs.	Spray	20	3	8/2, 8/19, 9/3	None	30-50	4	1,000	11.4	58
B	26 Eureka	{N. O. molasses / Cane sugar}	5 gals. / 25 lbs.	Spray	5	3	8/2, 8/19, 9/3	Moderate	30-50	4	1,139	13.9	49
C	26 Eureka	Check, no treatment	....	....		...		....	30-50	4	934	27.0	...

* Data obtained from count of nuts on Eureka trees (unless otherwise indicated) just prior to harvest (September 30).

† Peaches not treated.   ‡ Indicates effectiveness of A over B.

TABLE 27—*(Concluded)*

Experiment	Number of trees and variety	Application							Results				
		Material	Concentration (per 100 gals. for sprays and per 100 lbs. for dusts)	Method	Am't per tree, gals. for sprays and lbs. for dusts	Number	Date	Foliage injury "burn"	Estimated per cent infested 1928	Trees counted	Total nuts counted	Mean* per cent infested	Per cent control*
VII	(6 acres)												
A	85 Eureka	Basic lead arsenate / Table syrup§	6 lbs. / 5 gals.	Spray	¾‖	3	7/31, 8/16, 9/4	Moderate	20–40	20	2,018	1.3	35
B	17 Eureka	Check, no treatment							20–40	8	682	2.0	…
VIII	(1½ acres)												
A	13 Eureka	Basic lead arsenate	8 lbs.	Spray	15	2	8/4, 8/21	None	40–60	5	1,056	2.9	76‡
B	13 Eureka	Basic lead arsenate / Corn syrup "Karo"	8 lbs. / 5 gals.	Spray	15	2	8/4, 8/21	Moderate	40–60	5	1,000	11.9	…
IX	(7½ acres)												
A	43 Eureka	Basic lead arsenate	12 lbs.	Spray	6	3	8/1, 8/18, 9/16	None	20–40	4	1,341	5.8	…
B	85 Eureka	Basic lead arsenate / N. O. molasses / Beet sugar	6 lbs. / 5 gals. / 25 lbs.	Spray	3	3	8/1, 8/18, 9/16	Moderate	20–40	4	1,215	3.6	38**
X	(5 acres)												
A	42 Eureka	Basic lead arsenate / Corn sugar	16 lbs. / 25 lbs.	Spray	3	2	8/5, 8/24	Slight	30–50	4	1,121	1.5	…
	42 Franquette								30–50	4	1,015	0.8	…
	1 Klondike								70–90	1	175	0.5	…
XI	(10 acres)												
A	54 Eureka + 45 Neff	Basic lead arsenate / Corn sugar	16 lbs. / 25 lbs.	Spray	3	3	8/3, 8/17, 9/4	Slight	40–60	6	2,307	6.8	75
B	54 Eureka + 45 Neff	Basic lead arsenate	100 lbs.	Dust	1¾	3	8/3, 8/17, 9/4	None	40–60	6	2,531	3.5	87
C	12 Eureka + 10 Neff	Check, no treatment							40–60	6	2,114	27.0	…
	Totals— 27 plots, 1,238 walnut trees, 157 peach, 70 acres									161	38,952		

* Data obtained from count of nuts on Eureka trees (unless otherwise indicated) just prior to harvest (September 30).
† Indicates effectiveness of A over B.   § Probably made of unrefined sugar.   ‖ Small trees.   ** Indicates effectiveness of B over A.
‡ Indicates effectiveness of A over B.

If the material was effective in killing the flies, it is entirely possible that many of those flies that would have infested the walnuts in the check row were killed as a result of feeding during migratory stays on the treated foliage. The results indicated are of doubtful significance.

Plot Experiment II.—The data indicate that basic lead arsenate was more effective applied as a spray than as a dust (fig. 71). Approximately equal amounts of the actual toxic material should have been
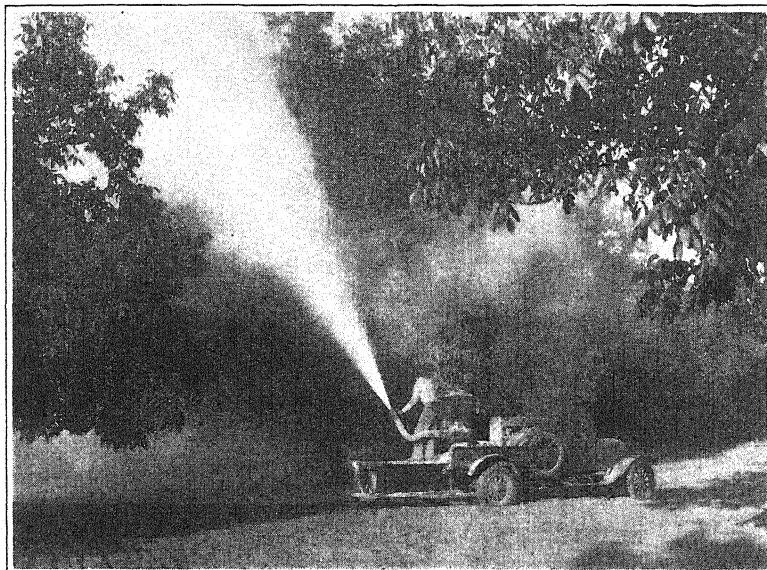
Fig. 71.—Dusting equipment in operation in experimental control plots.
(Photo taken at sunrise.)

retained by the tree in both plots. This result is contrary to results obtained in the laboratory toxicity studies.

Results indicate that a high degree of control was obtained in the spray plot.

Plot Experiment III.—This experiment was outlined to compare the efficacy of two and three applications of basic lead arsenate, with 10 and with 20 gallons per tree at each application. Results indicate that a high degree of control was obtained in all treated plots. The degree of infestation was too light in the treated plots to warrant comparison of the different treatments.

Plot Experiment IV.—The trees in this grove were relatively small and were interplanted with mature peach trees that were untreated. Results indicate that everything must be treated within a solid block to secure best results. Flies may reach egg-laying maturity on untreated

foliage, then migrate to treated trees and oviposit despite the presence of toxic materials.

Plot Experiment V.—This experiment was designed to determine whether or not the presence of sweetened material in the spray mixture increased the degree of control. The data indicate that it actually decreased the effectiveness of the lead arsenate, which is highly improbable. Flies were observed to feed freely on the spray material for several days after it was applied. This suggests variables that have not been accounted for. Slight foliage injury resulted where the syrup was used.

Plot Experiment VI.—This experiment was conducted to determine the merits of the bait spray as compared with full coverage of lead arsenate. The formula was similar to that used successfully in the control of the Mediterranean fruit fly with the exception of the substitution of basic lead arsenate for the acid lead arsenate. The sweetened material caused moderate foliage injury. Results indicate that the bait spray was inferior to the regular lead arsenate spray. The control in both plots was unsatisfactory.

Plot Experiment VII.—This was another test of the bait spray. Conclusions are unwarranted, because of the light infestation in both treated and check plots.

Plot Experiment VIII.—This experiment was designed to determine whether or not a higher degree of control could be obtained by increasing the dosage of lead arsenate, and also whether the addition of sweetened material resulted in still better control. The trees were of such size that 15 gallons of spray afforded satisfactory coverage. Results indicate superior performance of the lead arsenate where the sweetened material was omitted. This is apparently another instance of variables that are unaccounted for. Moderate foliage injury resulted from the use of the corn syrup.

Plot Experiment IX.—Data from this experiment indicate that 6 pounds of lead arsenate plus sweetened material, at 3 gallons per tree, is superior to 12 pounds of lead arsenate at 6 gallons per tree. This evidence is contradictory to that for experiments V, VI, and VIII, which show decreased control wherever sweetened material was used with lead arsenate; compare experiment VI B, which has the same formula as that used in IX B except that the former included cane sugar instead of beet sugar. In VI B the infestation was 13.9 per cent, while in IX B it was 3.6 per cent. Five gallons per tree was used in VI B, and 3 gallons per tree in IX B. Such erratic results are difficult to explain.

Plot Experiment X.—The dosage of basic lead arsenate was increased to 16 pounds to 100 gallons in this bait spray test. While a check was not maintained, the data indicate a high degree of control.

Plot Experiment XI.—The same material was used in plot A of this test as in experiment X with considerably poorer results. Straight basic lead arsenate dust produced a higher degree of control than did the bait spray, which contained 16 pounds of basic lead arsenate to 100 gallons. The data in this experiment appear significant; however the row of check trees unavoidably received more irrigation water than the remainder of the grove. This fact may have rendered the walnuts in this row more susceptible to oviposition.

Discussion of Field Control Experiments in 1929.—The generally erratic results obtained in the 1929 experiments afford very little reliable information; therefore only tentative conclusions may be drawn. However the following comments are justified: The degree of control obtained through the use of basic lead arsenate has been generally unsatisfactory. The addition of sweetened materials to the regular lead arsenate sprays did not increase their effectiveness. Bait sprays were no more promising than regular spray or dust applications of basic lead arsenate.

Plots of sufficient size and proper isolation are important features in obtaining significant data. Adequate check plots are indispensable in experiments of this nature. They can be dispensed with only when a reliable standard treatment has been developed, thereby enabling direct comparisons with other plots that are differentially treated.

The relation of irrigation practices to husk hardness at the time of maximum fly activity is not entirely understood. Since husk hardness appears quite definitely to be the most important factor governing susceptibility to infestation, it may have been partially responsible for the erratic results in these control studies.

The Use of Sweet Materials in Control Practices.—The success of bait sprays in Oregon in controlling the white-banded cherry fruit fly, *Rhagoletis cingulata* (Loew),[25] and also the use of sugar in population studies of *R. completa,* warranted investigation of the role of sweet materials. Chemotropic studies previously presented indicate that sucrose does not attract the walnut husk fly; however, when applied to the foliage, it does serve to congregate the flies; in moving about over the tree in search of food, they find the treated foliage and remain to engorge themselves. Fermenting New Orleans molasses offers some attraction. In population studies, 10 per cent sucrose solution sprayed onto small areas of foliage served to congregate the flies for only a few days. Therefore, tests were conducted to determine the length of time after application that sucrose and New Orleans molasses remain palatable to the fly.

TABLE 28

LENGTH OF TIME AFTER APPLICATION THAT SUCROSE AND NEW ORLEANS MOLASSES REMAIN PALATABLE*

Concentration, per cent	Days after application	R. completa observed feeding			Drosophilids observed feeding			Visible evidence of material on foliage			Injury to foliage			
		Many	Few	None	Many	Few	None	Plain	Faint	None	Severe	Moderate	Slight	None
	Granulated cane sugar spray													
5	1	×			×			×						×
	2–3		×		×				×					×
	4			×		×				×				×
	5–6			×			×			×				×
10	1	×			×			×						×
	2		×		×			×						×
	3		×		×				×					×
	4			×		×				×				×
	5–6			×			×			×				×
25	1–3	×			×			×						×
	4		×		×				×					×
	5			×		×			×				×	
	6			×		×			×			×		
50	1–2	×			×			×						×
	3	×			×			×					×	
	4	×				×		×				×		
	5		×			×			×			×		
	6			×			×			×	×			

* Observations extended from September 5 to 15, 1929.

TABLE 28—(Concluded)

Concentration, per cent	Days after application	R. completa observed feeding			Drosophilids observed feeding			Visible evidence of material on foliage			Injury to foliage			
		Many	Few	None	Many	Few	None	Plain	Faint	None	Severe	Moderate	Slight	None
Powdered cane sugar dust														
100	1	×			×			×						×
	2	×			×				×					×
	3	×			×					×				×
	4		×							×				×
	5			×		×				×		×		
	6			×		×				×				
New Orleans molasses spray														
10	1		×		×			×						×
	2		×		×			×					×	
	3–6			×	×				×				×	
25	1	×			×			×						×
	2		×			×		×					×	
	3–6			×		×			×			×		
50	1	×			×			×						×
	2		×		×			×				×		
	3					×		×				×		
	4–6			×		×			×		×			

Tests made were in the same grove and flies were present in large numbers. Two small areas of foliage were treated on each of two trees in each individual test. The treated areas were observed for the presence of flies at an optimum time twice daily. The data obtained from these tests are presented in table 28.

After a period of eight days had elapsed, the applications were repeated on areas contiguous to those formerly treated. The later observations collaborated those previously recorded, lending significance to the data.

With cane sugar, the period over which the material remains palatable to the flies was in direct proportion to the concentration used. At concentrations of 5 and 10 per cent, this period is of 2 or 3 days' duration. No foliage injury was evident in the span of these tests from these concentrations of sugar. However, tree-tolerance tests have shown that 3 to 5 per cent concentrations of sucrose do cause slight injury. The indications are that powdered cane sugar applied as dust offers more promise in studies of this nature than granulated cane sugar in solution at any concentration.

New Orleans molasses is apparently not particularly palatable to the flies until about 24 hours after it has been applied. Furthermore, it is effective for about 24 hours only. At 10 per cent concentration, only a few flies were observed feeding upon it; while at 25 and 50 per cent concentrations many flies were congregated. The injury factor incident to its use is important.

In most instances drosophilids were observed feeding on the treated foliage for several days after it had lost its effectiveness in serving to congregate walnut husk flies.

On the basis of these limited tests, the addition of cane sugar or molasses to sprays or dusts of basic lead arsenate is of questionable value in the control of *Rhagoletis completa*. Furthermore bait sprays consisting of either of these sweet materials and a more rapidly acting poison than basic lead arsenate, do not appear very promising. Semiweekly applications during the period of adult emergence would probably be necessary in order to effect a satisfactory degree of control.

Tree-Tolerance Studies.—The effect of various materials upon walnut trees was determined in conjunction with laboratory toxicity studies of the same materials on the flies. These tests were made on seven-year-old Eureka walnuts at the Citrus Experiment Station. Because of the relatively large number of tests planned and the desirability of maintaining buffer trees between treated plots, it was necessary to restrict the size of plots to one tree. However, only normal trees were included in the tests. Most materials were applied both as spray and dust, one, two, and

TABLE 29
TOLERANCE OF EUREKA WALNUT TREES TO CERTAIN INSECTICIDAL MATERIALS*

Material	Solubility in water at 20° C†	Concentration	Application Method‡	Application Number	Degree of injury§	Remarks
Magnesium fluosilicate	1 : 2.2	4 lbs.–100 gallons	Spray	1 2 3	Moderate Severe Severe	New growth shows first evidence of injury from fluorine compounds. Tips and margins of leaves, and also irregular areas between veins, have water-soaked appearance that later results in a "burned" appearance. Leaves of moderately to severely injured new growth usually drop, sections of twigs being defoliated, with new growth at the tips coming out after the treatment. All fluorines tested exhibited relatively poor sticking qualities. However, sodium fluoaluminate and potassium fluoaluminate apparently adhered better than any of the other fluorine compounds.
	1 : 2.2	33 per cent + talc	Dust	1	Slight	
Sodium fluoride	1 : 25	33 per cent + talc	Dust	1 2 3	Moderate Severe Severe	
		4 lbs.–100 gallons	Spray	1 2 3	Slight Moderate Severe	
Sodium fluosilicate 98–100 per cent	1 : 151	100 per cent (straight)	Dust	1 2 3	Slight Moderate Moderate	
		33 per cent + talc	Dust	1 2 3	Very slight Slight Moderate	
Sodium fluosilicate 70–75 per cent	1 : 151	4 lbs.–100 gallons	Spray	1 2 3	Very slight Moderate Severe	
		100 per cent (straight)	Dust	1 2 3	None Very slight Moderate	
		4 lbs.–100 gallons	Spray	1 2 3	Very slight Very slight Slight	
Sodium fluoaluminate (synthetic)‖	1 : 1,639	33 per cent + talc	Dust	1 2 3	None Very slight Slight	
		100 per cent (straight)	Dust	1	Slight	

See page 528 for footnotes for this table.

TABLE 29—(*Continued*)

Material	Solubility in water at 20° C†	Concentration	Application Method‡	Application Number	Degree of injury§	Remarks
Potassium fluoaluminate¶	1 : 633	20 per cent + talc...............	Dust	2	None	
Barium fluoride...............	1 : 632	(4 lbs.–100 gallons...............	Spray	1, 2, 3	None, None, Very slight	
		33 per cent + talc...............	Dust	1, 2, 3	None, None, Very slight	
Barium fluosilicate...............	1 : 4,000	(4 lbs.–100 gallons...............	Spray	1, 2, 3	None, Very slight, Slight	
		33 per cent + talc...............	Dust	1, 2, 3	None, None, Very slight	See "Remarks," preceding page
		100 per cent (straight)...............	Dust	1	Slight	
Potassium fluosilicate...............	1 : 1,130	33 per cent + talc...............	Dust	1, 2	None, Very slight	
		(4 lbs.–100 gallons...............	Spray	1, 2, 3	None, None, Very slight	
Calcium fluosilicate compound...............	1 : 25,636	100 per cent (straight)...............	Dust	1, 2, 3	None, None, Very slight	
		(4 lbs.–100 gallons...............	Spray	1, 2, 3	None, None, Very slight	
Calcium fluoride...............	1 : 57,200	33 per cent + talc...............	Dust	1, 2, 3	None, None, Very slight	

See page 528 for footnotes for this table.

TABLE 29—(Continued)

Material	Solubility in water at 20° C†	Concentration	Application — Method‡	Application — Number	Degree of injury§	Remarks
Sodium arsenite	Very soluble	2 quarts–100 gallons	Spray	1	Very severe	Total defoliation and defruiting of trees.
		1 quart–100 gallons	Spray	1	Very severe	
Calcium arsenate	1 : 133	2 lbs.–100 gallons	Spray	1	Slight	
				2	Moderate	
				3	Severe	
		33 per cent + lime	Dust	1	Slight	
Acid lead arsenate	1 : 200	3 lbs.–100 gallons	Spray	1	Moderate	
				2	Severe	
				3	Severe	
		100 per cent (straight)	Dust	1	Slight	Typical arsenical injury is manifested in irregular burned areas between veins and along midrib of new and mature foliage. Moderately to severely injured leaves drop. Yellowing of leaves partially or totally, resulting in drop, also appears to be related to arsenical injury.
				2	Severe	
				3	Severe	
Basic lead arsenate	1 : 200	4 lbs.–100 gallons	Spray	1	None	
				2	None	
				3	None	
		6 lbs.–100 gallons	Spray	1	None	
		12 lbs.–100 gallons	Spray	1	None	
		100 per cent (straight)	Dust	1	None	
				2	None	
				3	Very slight	
Magnesium arsenate¶	1 : 200	3 lbs.–100 gallons	Spray	1	None	
		20 per cent + lime	Dust	2	None	
Manganese arsenate¶	1 : 200	3 lbs.–100 gallons	Spray	1	Slight	
		20 per cent + lime	Dust	1	Slight	
Copper sulfate‖	1 : 5	4 lbs.–100 gallons	Spray	1	Moderate	Copper injury is manifested in moderate to large water-soaked areas between the veins occuring on both new and mature foliage. Affected leaves drop. Burn of margin and tip of leaf common but negligible in consequence.
				2	Severe	
				3	Very severe	
		10 per cent + lime	Dust	1	Slight	
				2	Moderate	
		20 per cent + lime	Dust	1	Slight	
				2	Moderate	

See page 528 for footnotes for this table.

TABLE 29—(*Concluded*)

Material	Solubility in water at 20° C†	Concentration	Application Method‡	Application Number	Degree of injury§	Remarks
Copper carbonate‖	Insoluble	(4 lbs.-100 gallons	Spray	{1, 2, 3	Slight / Severe / Severe	See "Remarks" opposite "copper sulfate" on preceding page.
		20 per cent + lime	Dust	{1, 2, 3	Slight / Slight / Moderate	
		90 per cent + lime	Dust	1	Severe	
Cane sugar (powdered)		100 per cent (straight)	Dust	1	Moderate	
Beet sugar		25 lbs.-100 gallons	Spray	1	Slight	Typical injury from sweetened materials is manifested in a burning of leaf tips and margins, also large, irregular areas between veins. Leaves commonly turn yellow and drop without showing much evidence of being burned.
"Black Strap Molasses"		40 lbs.-100 gallons	Spray	1	Slight	
Beet sugar / "Black Strap Molasses"		25 lbs. / 40 lbs. } -100 gallons	Spray	1	Moderate	
Basic lead arsenate / Beet sugar / "Black Strap Molasses"		6 lbs. / 25 lbs. / 40 lbs. } -100 gallons	Spray	1	Moderate	
Basic lead arsenate / Beet sugar		6 lbs. / 25 lbs. } -100 gallons	Spray	1	Slight	
Basic lead arsenate / "Black Strap Molasses"		6 lbs. / 40 lbs. } -100 gallons	Spray	1	Slight	

* All tests were in 1929 except where otherwise indicated.

† Solubilities of most fluorine compounds tested were determined by the Division of Chemistry, California State Department of Agriculture.

‡ Application was thorough in every instance. Subsequent applications were made at 2-week intervals.

§ All plots were carefully inspected weekly to determine effect of material on trees. Degree of injury is designated as follows:
    Very slight—Trace of leaf injury.
    Slight—Burn of tips and margins of leaves quite general over entire tree.
    Moderate—Approximately one-fourth of total leaf area killed
    Severe—Approximately one-half of total leaf area killed.
    Very severe—Practically total defoliation.

‖ Tests conducted in 1930.

¶ Tests conducted in 1932.

three applications being made. Data obtained from these tests in 1929, together with data from tests of several other materials in 1930 and 1932, are presented in table 29.

The more soluble fluorine compounds were so injurious that they were eliminated from further trials. Considering tree tolerance, availability, and existing information regarding toxicity, barium fluosilicate and synthetic cryolite are more promising than any other fluorine compounds tested.

Tests of arsenicals corroborated conclusions of other workers in that basic lead arsenate is the only one of the commonly used arsenical compounds that can be applied with safety to walnut trees. However on the basis of one season's (1932) tests, magnesium arsenate appears promising with respect to safety to walnut foliage.

Copper sulfate and copper carbonate caused slight to severe injury at the concentrations tested. This is not entirely in accord with observations of phytopathologists.

The sweet materials tested resulted in slight to moderate injury. Studies previously reported indicate the questionable value of these materials when included in insecticide mixtures at the concentrations tested. No more injury was produced by the combination of sweet with basic lead arsenate than by the sweet material alone.

FIELD INVESTIGATIONS IN 1930

In view of the somewhat erratic results obtained in 1929, an effort was made in 1930 to isolate all plots from each other and from surrounding vegetation. Furthermore this was desirable particularly where materials were applied as dust, since there is a certain amount of drift into adjoining trees, even under ideal conditions for application. Consequently four rows of trees surrounding each plot were sprayed with basic lead arsenate. This was considered standard treatment. These treated rows are hereafter referred to as "contact" areas. Figure 72 illustrates the typical method used in isolating plots (though these particular plots were not used in 1930).

More detailed attention was given to the fly population in the various plots in 1930. The basis of rating fly population numerically was not entirely satisfactory; however, it served as an index, at least, of the relative numbers of flies in various plots. The procedure was as follows:

When emergence records indicated that 50 to 75 per cent of the seasonal emergence had occurred, the rating of plots was begun. A 10 to 20 per cent solution of cane sugar was sprayed onto a small area on the northeast portion of a representative number of trees in each plot. The treated areas were in a position such that all flies present could be

readily observed. The time of observation with respect to temperature, humidity, and sunlight was comparable and, therefore, the records appear to be significant.

The arbitrary classes of 1, 2, 3, and 3+ were established to indicate the population found to exist as a result of these field studies. When the mean number of flies observed per tree was one, the rating of 1 was given; when from two to three, the rating of 2; when from four to five, the rating of 3; and when more than five, the rating of 3+.

There were no heavily infested groves in 1929, so that the experimental plots did not have a potentially high fly population from the annual generation. However, all plots were moderately to heavily infested in 1928. Since emergence in 1929 was only approximately 45 per cent of the total, the population potential of the biennial generations was relatively high.

Data regarding infestation in these control studies were based on field counts just prior to harvest. The "count trees" were located centrally within plots. Individual tree records were kept to enable statistical treatment of the data. For calculating probable error of the mean, the Gaussian formula was used in those instances where the number of count trees was 16 or more. Since in most tests the number of count trees was under 16, the Bessel formula was used for most of the calculations. These standard formulas are as follows:[20]

$$\text{Gaussian: P.E.} = \pm 0.6745 \times \frac{\sigma}{\sqrt{N}}$$

$$\text{Bessel:} \quad \text{P.E.} = \pm \frac{0.6745 \times \sigma}{\sqrt{N-1}}$$

Adult emergence began in the middle of July and the seasonal peak was reached shortly thereafter on July 18 (fig. 39). Plots were differentially treated; however, in all instances one application was made before the peak was reached. The data obtained from these field control experiments are presented in table 30.

Plot Experiment I.—This experiment was outlined to determine the efficacy of one application of basic lead arsenate spray as compared with that of two applications. Since differences in percentage of infestation between untreated plots varied as greatly as those between treated and untreated plots, important conclusions are unwarranted. However, the percentages of infestation in plots B and C indicate a superiority of two spray applications over one application.

Plot Experiment II.—It was desirable to determine the relative merits of basic lead arsenate applied as spray and as dust; also the effect of a single application just prior to the peak of adult emergence. Concerning the latter, the application in plot B was made on August 12, in the

TABLE 30

CONTROL EXPERIMENTS IN 1930

Experiment	Number of trees and variety	Material	Concentration	Application				Estimated per cent infested 1929*	Population rating†	Results		
				Method	Amount per tree	Number	Date			Trees counted	Total nuts counted	Mean per cent infested‡
I	(10 acres)											
A	25 Eureka	Basic lead arsenate	4 lbs.–100 gals.	Spray	15 gals.	1	7/17	1–2	1	4	2,104	2.2±0.6
B	24 Eureka	Basic lead arsenate	4 lbs.–100 gals.	Spray	15 gals.	2	7/17, 8/3	1–2	2	4	2,240	0.9±0.6
C	92 Eureka§	Basic lead arsenate	4 lbs.–100 gals.	Spray	15 gals.	2	7/17, 8/3	1–2	2	4	2,342	1.0±0.6
D	24 Eureka	Check, no treatment						1–2	2	4	1,933	2.0±0.5
E	24 Eureka	Check, no treatment						1–2	2	4	2,193	6.2±1.8
D-E								1–2	2	8	4,126	4.2±1.1
II	(10 acres)											
A	32 Eureka	Basic lead arsenate	4 lbs.–100 gals.	Spray	15 gals.	1	7/16	4–6	2	4	2,141	6.2±1.0
B	32 Eureka	Basic lead arsenate	4 lbs.–100 gals.	Spray	15 gals.	1	8/12	4–6	1	4	2,160	3.8±0.8
C	32 Eureka	{Basic lead arsenate / Hydrated lime	20 lbs. / 80 lbs.}	Dust	3 lbs.	1	7/16	4–6	2	4	2,152	12.2±3.7
D	32 Eureka	{Basic lead arsenate / Hydrated lime	20 lbs. / 80 lbs.}	Dust	3 lbs.	2	7/16, 8/13	4–6	3	4	2,209	6.4±1.4
E	95 Eureka§	Basic lead arsenate	4 lbs.–100 gals.	Spray	15 gals.	2	7/16, 8/13	4–6	2	4	1,721	2.5±0.6
III	(3¼ acres)											
A	12 Eureka + 42 peach	Walnuts: Basic lead arsenate.	4 lbs.–100 gals.	Spray	15 gals.	3	7/16, 8/3, 8/20	4–6	3	4	2,446	11.5±1.2
A1	20 Eureka + 51 peach	Peaches: {Basic lead arsenate / Powdered cane sugar	9 lbs. / 1 lb.}	Dust	1½ oz.	2	7/18, 8/5	4–6	2	4	1,533	11.1±1.4
A-A1									3	8	3,979	11.3±0.7
B	12 Eureka + 42 peach	Walnuts: Basic lead arsenate	4 lbs.–100 gals.	Spray	15 gals.	3	7/16, 8/3, 8/20	4–6	3	4	1,431	11.7±2.6
B1	18 Eureka + 48 peach	Peaches: check, no treatment						4–6	3	4	2,010	17.8±0.9
B-B1								4–6	3	8	3,441	15.2±1.6

* All plots were moderately to heavily infested in 1928; thus the biennial brood would greatly augment the fly population in 1930.

† See pp. 520–530 for method of determining population rating.

‡ Data based on count of nuts on trees just prior to harvest (October 10); percentage infested with walnut husk fly except where otherwise indicated.

§ These were trees surrounding experimental plots for purpose of isolation, called "contact trees" in the text.

TABLE 30—(Concluded)

Experiment	Number of trees and variety	Material	Application					Estimated per cent infested 1929*	Population rating†	Results		
			Concentration	Method	Amount per tree	Number	Date			Trees counted	Total nuts counted	Mean per cent infested‡
IV (5 acres)¶												
A	85 Eureka	Basic lead arsenate	4 lbs.–100 gals.	Spray	10 gals.	1	7/17	4–6	1	16	2,116	3.9±0.3
A1	20 Eureka	{Basic lead arsenate / Powdered cane sugar}	{9 lbs. / 1 lb.}	Dust	3 oz.	2	7/18, 8/17	4–6	1	7	1,178	5.3±0.6
A2	28 Eureka	Check, no treatment						4–6	2	15	1,422	2.3±0.4
B	35 Payne	{Basic lead arsenate / Powdered cane sugar}	{9 lbs. / 1 lb.}	Dust	3 oz.	2	7/18, 8/17	2–4	2	14	1,751	0.0
B1	10 Payne	Check, no treatment						2–4	1	16	1,782	0.1±0.0
V (12 acres)‖												
A	24 walnut	Basic lead arsenate	4 lbs.–100 gals.	Spray	5 gals.	2	7/17, 8/12	10–30	2	**	....	15.5††
B	24 walnut	Basic lead arsenate	4 lbs.–100 gals.	Spray	10 gals.	2	7/17, 8/12	10–30	1	...	....	35.0††
C	48 walnut	{Barium fluosilicate‡‡ / Talc}	{20 lbs. / 80 lbs.}	Dust	2 lbs.	2	7/16, 8/14	10–30	1	...	....	3.4††
D	128 walnut§	Basic lead arsenate	4 lbs.–100 gals.	Spray	15 gals.	2	7/17, 8/12	10–30	1	...	....	
E	24 walnut	Check, no treatment						10–30	2	...	....	12.8††
VI (10 acres)												
A	42 Eureka	{Barium fluosilicate‡‡ / Talc}	{20 lbs. / 80 lbs.}	Dust	2 lbs.	2	7/18, 8/13	1–2	1	10	4,154	0.9±0.2
B	10 Eureka	{Basic lead arsenate / Hydrated lime}	{20 lbs. / 80 lbs.}	Dust	2 lbs.	2	7/18, 8/13	2–4	1	2	449	36.7±6.6
C	70 Eureka§	Basic lead arsenate	4 lbs.–100 gals.	Spray	15 gals.	2	7/16, 8/12	1–2	1	...	....	
D	42 Eureka	Check, no treatment						1–2	1	9	2,621	8.3±1.9
Totals, 27 plots, 1,068 walnut trees, 183 peach trees, 51 acres										145	44,088	

* All plots were moderately to heavily infested in 1928; thus the biennial brood would greatly augment the fly population in 1930.

† See pp. 529-530 for method of determining population rating.

‡ Data based on count of nuts on trees just prior to harvest (October 10); percentage infested with walnut husk fly except where otherwise indicated.

§ These were trees surrounding experimental plots for purpose of isolation, called "contact trees," in the text.

¶ Small trees.

‖ Assorted varieties of large trees; grove proved unadapted for desired experiments.

** Not practicable to make counts since trees are very old and tall, and pruned high.

†† Percentage of harvest culls, including nuts injured by walnut husk fly, blight, sunburn, and codling moth.

‡‡ Slight foliage injury, though negligible.

belief that the seasonal peak of emergence was yet to be reached, while actually more than 70 per cent of the total emergence had taken place by that date. The data indicate results superior to one application made just prior to the peak, and also superior to two applications of the material as dust. A possible explanation lies in the fact that the material was more readily available to a larger percentage of the total flies, since it was applied later and thus was not covered with dust from the soil as a result of orchard cultivation. This test shows that appreciable oviposition did not take place, at least within 30 days after the seasonal peak of emergence—a fact of interest and importance.

These tests demonstrate that under existing conditions basic lead arsenate is more efficacious when applied as spray than as dust.

Plot Experiment III.—This experiment was designed to determine the value of treating trees other than walnuts that are growing as interplants. All walnut trees were sprayed with basic lead arsenate at the regularly used concentration and coverage. Peach trees in half of the grove were treated with a poison bait, which was applied mainly to the nonbearing sucker growth in the centers of the trees. Peach trees in the remaining half of the grove were untreated and served as a check. The results indicate a slight reduction in the infestation where the peaches were treated. Considering the results obtained in other experiments with two spray applications of basic lead arsenate, it is evident that the material applied to the peach trees was of little value. Furthermore the indications are that the faliure to effect a higher degree of control on walnuts is directly attributable to the fact that the peaches did not receive effective treatments.

Plot Experiment IV.—This experiment was conducted to compare the efficacy of basic lead arsenate used in a "bait" dust, with that applied as spray with complete coverage. The results afford no information regarding control, but they do point out the nature of some of the uncontrollable variations—for instance, a higher degree of infestation occurred in the tested plots A and A1 than in the check A2, while a higher fly population existed in plot A2.

Plot Experiment V.—The trees in this grove were assorted varieties, which vary in susceptibility to attack. Besides, they were old, tall, and pruned high; and on the whole were not entirely satisfactory for experimental use. The purpose of the experiment was to determine the efficacy of a very light spray application of basic lead arsenate, and of barium fluosilicate applied as dust. The percentage of infestation could not be satisfactorily determined in the field; therefore the percentage of cull walnuts in each plot was obtained from the harvest record. While these data are not truly signficant with respect to the degree of infestation of

the walnut husk fly, they indicate roughly the relative effectiveness of the various treatments. Barium fluosilicate apparently effected a considerable reduction in the degree of infestation. This material caused slight foliage injury, particularly to the newest growth, although the injury was negligible in extent. A light application of lead arsenate is apparently of little value in controlling the fly.

Plot Experiment VI.—This experiment was a comparison of barium fluosilicate and basic lead arsenate applied as dust. As evidenced by the results obtained, barium fluosilicate is greatly superior to basic lead arsenate. Slight foliage injury resulted from the use of the former material; however, it was negligible in degree.

The data from this experiment illustrate how the degree of infestation may vary within a grove. For instance, the infestation in plot B, after two dust applications, was 36 per cent; while in plot D, which was not treated, the infestation was 8 per cent.

Discussion of Field Control Experiments in 1930.—The fact that in most instances the probable error of the mean percentage infested is less than one-third of the uncorrected datum, indicates significance of the data.

In summarizing the results obtained from the various control experiments, the following conclusions appear justifiable: Two spray applications of basic lead arsenate, 4 pounds to 100 gallons, offer a fairly satisfactory means of controlling the walnut husk fly. The efficacy of this material applied as a spray is superior to dust applications; in fact, one spray treatment is approximately equivalent to two dust treatments. Barium fluosilicate is sufficiently promising to warrant further investigation. For the best results, all interplanted trees and other vegetation within the walnut grove proper, together with that immediately bordering the outside rows, should be treated. Isolation of experimental plots is essential. The method employed for this purpose in these studies is satisfactory and practicable. Accurate comparisons of results obtained from the use of the same materials in different groves are not possible, because of differences in soil types, irrigation, and other cultural practices, as well as the size of the crop of nuts that is developing. When fly populations are comparable, the latter feature apparently bears some relation to percentage infestation.

The fact that in most instances the population rating of treated plots was practically the same as that of untreated plots suggests that the method of obtaining this index did not furnish detailed information with a high degree of accuracy. Therefore, its use is limited and conclusions based thereon require qualification. However, on the assumption that the method is reasonably reliable, there apparently is no signifi-

cant relation between the number of flies present in a plot and the resulting percentage of infested nuts. If true, it is logical to suspect that not all of the walnuts were in an optimum condition for females to puncture the husks in oviposition. In this connection, with reference to experiment I, notes on observations of the abundance of flies present in 1928 indicate that they were no more plentiful then than during the 1930 season. In 1928 the infestation was estimated to be between 70 and 90 per cent; while in 1930 it did not exceed 6 per cent in any of the plots.

FIELD INVESTIGATIONS IN 1931

In the field plot trials for 1931 virtually the same procedure was followed as in the 1930 studies except that the contact areas were sprayed with barium fluosilicate instead of basic lead arsenate. However, in some respects more detailed information was desirable. This information regarding history of the plots in 1930 seemed necessary as an aid in planning experiments and laying out plots for the 1931 control studies. In many of the experiments actual tree counts of the number of infested nuts per tree in 1930 were made in order to obtain an idea of the homogeneity of the infestation. It was not practicable to obtain this information in all groves, nor was the percentage of infestation determined in some instances where counts were made of infested nuts per tree. Furthermore, in some plots the percentage of infestation was estimated. Where data are given for individual plots these are relative to other plots within the experiment.

A larger number of count trees were used in the 1931 experiments than in previous studies. Since control plots were maintained in most instances, the apparent percentage of reduction in infestation (or percentage of control), resulting from the use of the various materials, was determined. For these computations the commonly used formula discussed in the 1929 control studies was employed. However, as used in the 1931 studies $\bar{A}$ and $\bar{B}$ are both affected by probable errors. Therefore it was necessary to calculate the probable error of the quotient $\dfrac{\bar{B} \pm b}{\bar{A} \pm a}$. In these instances the following formula was used:[20]

$$Eq = \pm \frac{\sqrt{\left(\dfrac{\bar{B}a}{\bar{A}}\right)^2 + b^2}}{\bar{A}}$$

Adult emergence began during the first week of July and the seasonal peak was reached a few days afterward on July 12 (fig. 42). Thus emergence was earlier than in any of the preceding seasons of this study.

The data obtained from these field control plots are summarized in table 31.

TABLE 31
CONTROL EXPERIMENTS IN 1931   *Columns continued on next page*

Experiment	Number of trees and variety	Material	Concentration (per 100 gals. for sprays and per 100 lbs. for dusts)	Method of application	Amount per tree, gals. for sprays and lbs. for dusts
I	(44 acres) Peach interplants				
A	48 Eureka + 48 peach	{ Barium fluosilicate / Talc	20 lbs. } 80 lbs. }	Dust	{ W[c] 2 } P[c] ½ }
B	48 Eureka + 48 peach	{ Barium fluosilicate / Diatomaceous earth / Fish oil[e]	20 lbs. } 80 lbs. } 2 pts. }	Dust	{ W 2 } P ½ }
C	48 Eureka + 48 peach	{ Barium fluosilicate / Talc	20 lbs. } 80 lbs. }	Dust	{ W 2 } P ½ }
D	48 Eureka + 48 peach	Barium fluosilicate	3 lbs.	Spray	{ W 15 } P 5 }
E	48 Eureka + 48 peach (peaches not treated)	Barium fluosilicate	3 lbs.	Spray	{ W 15 } P 0 }
F	416 Eureka[f] + 416 peach[f]	Barium fluosilicate	3 lbs.	Spray	{ W 15 } P 5 }
G	48 Eureka + 48 peach	Check No. 1, no treatment	....	....	....
H	48 Eureka + 48 peach	Check No. 2, no treatment	....	....	....
II	(7 acres) Placentia interplants				
A	12 Eureka + 12 Placentia	{ Barium fluosilicate / Talc	20 lbs. } 80 lbs. }	Dust	3
B	12 Eureka + 12 Placentia	{ Barium fluosilicate / Talc	20 lbs. } 80 lbs. }	Dust	3
C	12 Eureka + 12 Placentia (Placentias not treated)	{ Barium fluosilicate / Talc	20 lbs. } 80 lbs. }	Dust	3
	12 Eureka + 12 Placentia (Placentias not treated)	{ Barium fluosilicate / Talc	20 lbs. } 80 lbs. }	Dust	3
E	18 Eureka + 12 Placentia	Check, no treatment	....	....	....
III	(5½ acres) Small walnut trees, peach interplants				
A	34 Eureka + 112 peach	Barium fluosilicate	3 lbs.	Spray	{ W 7 } P 5 }
B	36 Eureka + 112 peach (peaches not treated)	Barium fluosilicate	3 lbs.	Spray	{ W 7 } P 0 }
C	24 Eureka + 72 peach	Check, no treatment	....	....	....

[c] W = Walnut; P = peach.

[e] Fish oil was classified as "light-pressed."

[f] These were trees surrounding experimental plots for purpose of isolation, called "contact trees" in text.

TABLE 31—(*Continued*)

Number of applications	Date	Foliage injury "burn"	Average infestation, 1930		Population rating[a]	Results[b]			Per cent control
			Nuts per tree	Per cent		Trees counted	Total nuts counted	Mean per cent infested	
1	7/9	{W Slight[d] / P None}	17	....	2	28	4,182	16.9±1.0	45.0± 4.5
1	7/14	{W Slight / P None}	7	....	1	27	8,189	10.3±2.2	66.5± 7.0
2	7/9, 8/17	{W Slight / P None}	5	....	1	27	7,299	2.7±0.2	91.2± 4.5
1	7/14	{W Slight / P None}	3	....	1	26	9,952	6.2±0.4	79.8± 4.5
1	7/14	{W Slight / P None}	4	....	1	27	8,237	17.1±1.1	44.3± 4.5
1	7/18	{W Slight / P None}	3	....	1	12	4,460	5.9±0.4	80.8± 4.5
....	....	....	9	....	2	28	6,924	30.7±1.8	....
....	....	....	1	....	1	28	10,238	6.9±0.8	....
1	7/15	Slight			1	12	1,930	3.6±0.7	89.0± 4.3
2	7/15, 8/18	Slight			1	12	2,129	4.8±0.6	85.3± 4.3
1	7/15	Slight		5[g]	1	12	1,744	8.4±0.7	74.3± 4.3
2	7/15, 8/18	Slight			1	12	2,235	3.2±0.5	90.2± 4.3
....	....	....			3	18	2,970	32.6±2.4	....
1	7/10	Slight	19	65	1	33	1,128	2.2±0.4	90.4± 6.2
1	7/10	Slight	22	51	1	35	726	2.6±0.5	88.7± 6.2
....	....	....	15	55	2	21	418	22.9±1.9	....

[a] See pp. 527-530 for method of determining population rating.
[b] Data based on count of nuts on trees just prior to harvest (September 28).
[d] "Slight"—injury is negligible.
[g] Estimated average for entire grove.

TABLE 31—*(Continued)*

Experiment	Number of trees and variety	Material	Concentration (per 100 gals. for sprays and per 100 lbs. for dusts)	Method of application	Amount per tree, gals. for sprays and lbs. for dusts
IV A	(3½ acres) 30 Eureka	{ Barium fluosilicate { Talc	20 lbs. 80 lbs.	Dust	3
B	30 Eureka	{ Basic lead arsenate { Hydrated lime	20 lbs. 80 lbs.	Dust	3
V A	(12 acres) Placentia interplants 40 Eureka + 40 Placentia	{ Barium fluosilicate { Fish oil[e]	3 lbs. 1 pt.	Spray	10
B	30 Eureka + 30 Placentia	Hydrated lime	100 lbs.	Dust	3
C	30 Eureka + 30 Placentia	Check, no treatment			
VI A	(2½ acres) 3 Eureka (isolated in Placentia grove)	Barium fluosilicate	3 lbs.	Spray	15
B	4 Eureka + 32 Placentia (Eurekas isolated in Placentia grove—barrier of Placentias 1 tree deep treated surrounding individual trees)	Barium fluosilicate	3 lbs.	Spray	15
C	4 Eureka (isolated in Placentia grove)	Check, no treatment			
VII A	(2 acres) 2 Eureka (isolated in Placentia grove)	Cryolite (synthetic)	3 lbs.	Spray	15
B	3 Eureka + 24 Placentia (Eurekas isolated in Placentia grove—barrier of Placentias 1 tree deep treated surrounding individual trees)	Cryolite (synthetic)	3 lbs.	Spray	15
C	3 Eureka (isolated in Placentia grove)	Check, no treatment			
VIII A	(2½ acres) 3 Eureka (isolated in Placentia grove)	{ Cryolite (natural) { Diatomaceous earth { Fish oil[e]	20 lbs 80 lbs. 2 lbs.	Dust	2
B	4 Eureka + 32 Placentia (Eurekas isolated in Placentia grove—barrier of 1 tree deep treated surrounding individual trees)	{ Cryolite (natural) { Diatomaceous earth { Fish oil[e]	20 lbs. 80 lbs. 2 lbs.	Dust	2
C	4 Eureka (isolated in Placentia grove)	Check, no treatment			

[a] See pp. 529–530 for method of determining population rating.

[b] Data based on count of nuts on trees just prior to harvest (September 28).

[e] Fish oil was classified as "light-pressed."

TABLE 31—*(Continued)*

Number of applications	Date	Foliage injury "burn"	Average infestation, 1930		Population rating[a]	Results[b]			Per cent control
			Nuts per tree	Per cent		Trees counted	Total nuts counted	Mean per cent infested	
1	7/10	Slight	65	....	3	30	2,052	58.9±1.8	28.9± 3.4[h]
1	7/10	None	29	....	3	30	2,499	82.8±2.2	....
1	7/14	Slight			1	39	2,193	3.1±0.3	91.7± 3.8
1	7/13	None	....	5[g]	1	26	746	7.1±1.6	80.9± 5.9
....	....	....			2	24	772	37.1±1.9	....
1	7/10	Slight			3	3	792	39.0±7.3	38.6±13.7
1	7/10	Slight	....	50[i]	2	4	1,432	26.5±6.8	58.3±12.9
....	....	....			3	4	868	63.5±8.5	....
1	7/14	Slight			1	2	236	13.1±5.5	77.7±10.7
1	7/14	Slight	....	40[i]	1	3	179	31.2±6.2	46.8±13.3
....	....	....			2	3	319	58.6±8.7	....
1	7/14	Slight			2	3	656	59.6±5.0	11.6±12.9
1	7/14	Slight	....	50[i]	2	4	1,296	68.1±4.7	+1.0±14.1[j]
....	....	....			2	4	575	67.4±7.7	....

[g] Estimated average for entire grove.
[h] Indicates increased effectiveness of barium fluosilicate over basic lead arsenate.
[i] Estimated average for all Eureka trees in grove.
[j] Actual increase in degree of infestation over check.

TABLE 31—*(Continued)*

Experiment	Number of trees and variety	Material	Concentration (per 100 gals. for sprays and per 100 lbs. for dusts)	Method of application	Amount per tree, gals. for sprays and lbs. for dusts
IX	(10 acres) Neff interplants				
A	16 Eureka + 16 Neff	Cryolite (synthetic)............	20 lbs.	Dust	3
		Talc..............................	80 lbs.		
B	15 Eureka + 16 Neff...........	Cryolite (natural)...............	20 lbs.	Dust	3
		Talc..............................	80 lbs.		
C	15 Eureka + 16 Neff...........	Talc..............................	100 lbs.	Dust	3
D	42 Eureka [f] + 42 Neff [f]	Barium fluosilicate............	3 lbs.	Spray	15
E	16 Eureka + 16 Neff...........	Check, no treatment.........	....	....	....
X	(2 acres) Franquette interplants				
A	6 Franquette + 12 Placentia...........	Cryolite (synthetic)............	100 lbs.	Dust	2
B	4 Franquette + 10 Placentia...........	Check, no treatment.........	....	....	....
XI	(1½ acres) Eureka interplants				
A	4 Eureka + 16 Placentia............	Nicotine sulfate 40%...........	2½ pts.	Spray	15
		Butanol..........................	2½ pts.		
		White oil 80 sec. visc.........	3 gals.		
B	4 Eureka........................	Check, no treatment.........	....	....	....
XII	(3 acres)				
A	20 Eureka.......................	Nicotine sulfate 40%...........	1 pt.	Spray	15
		Tannic acid.....................	3 lbs.		
B	15 Eureka.......................	Barium fluosilicate............	3 lbs.	Spray	15
C	12 Eureka.......................	Check, no treatment.........	....	....	....
XIII	(3 acres) Eureka interplants				
A	12 Payne + 6 Eureka...........	Nicotine sulfate 40%...........	1 pt.	Spray	15
		Sucrose.........................	12 lbs.		
B	10 Payne + 9 Eureka...........	Nicotine sulfate 40%...........	5 lbs.	Dust	3
		Bentonite.......................	95 lbs.		
C	5 Payne + 5 Eureka...........	Check, no treatment.........	....	....	....

[a] See pp. 529-530 for method of determining population rating.

[b] Data based on count of nuts on trees just prior to harvest (September 28).

[f] These were trees surrounding experimental plots for purpose of isolation, called "contact trees" in text.

[i] Estimated average for all Eureka trees in grove.

TABLE 31—*(Continued)*

Number of applications	Date	Foliage injury "burn"	Average infestation, 1930		Population rating[a]	Results[b]			Per cent control
			Nuts per tree	Per cent		Trees counted	Total nuts counted	Mean per cent infested	
1	7/13	Slight	66	12	1	16	2,954	8.5±1.5	62.4± 9.7
2	7/13, 8/17	Slight	33	6	1	15	2,165	3.5±0.5	84.5± 6.2
2	7/13, 8/1	None	20	4	2	15	2,543	29.2±3.4	+29.0±22.0[j]
1	7/12	Slight	11	3	1	5	572	12.5±3.6	44.7±19.8
....	....	....	36	6	2	16	2,513	22.6±3.2	....
1	7/15	Slight	...	75[k]	1	6	223	1.7±0.4	....
....	....	....			1	4	257	0.0	....
2	7/15, 8/3	Moderate	....	60[i]	2	4	812	78.0±5.5	+15.0±17.4[j]
....	....	....			3	4	575	67.4±8.5	....
2	7/13, 8/5	None	....	30[i]	1	10	2,586	13.2±1.8	74.4± 5.3
2	7/13, 8/5	Slight			1	8	2,082	8.2±2.6	84.1± 6.1
....	....	....			3+	9	2,693	51.4±8.8	....
2	7/11, 8/2	Slight			3	13	2,685	44.4±6.0	45.3± 8.0
2	7/12, 8/5	None	....	40[l]	1	19	3,070	39.2±4.8	51.7± 6.9
....	....	....			3	7	1,950	81.1±6.0	....

[j] Actual increase in degree of infestation over check.
[k] Estimated average for all Franquette trees in grove.
[l] Estimated average for all Payne and Eureka trees in grove.

TABLE 31—*(Concluded)*

Experiment	Number of trees and variety	Material	Concentration (per 100 gals. for sprays and per 100 lbs. for dusts)	Method of application	Amount per tree, gals. for sprays and lbs. for dusts
XIV *A*	(5 acres) 20 Eureka + 19 seedling	{Nicotine sulfate 40% Diatomaceous earth	5 lbs.} 95 lbs.}	Dust	2
B	20 Franquette	{Barium fluosilicate Talc	20 lbs.} 80 lbs.}	Dust	2
C	15 Franquette + 5 Eureka	Check, no treatment	....	....	....
XV *A*	(10 acres) 25 Eureka	Tobacco dust	100 lbs.[t]	Dust	2
B	25 Eureka	Diatomaceous earth	100 lbs.	Dust	2
C	25 Eureka	Barium fluosilicate	100 lbs.	Dust	1¼
D	70 Eureka[f]	Barium fluosilicate	3 lbs.	Spray	20
E	25 Eureka	Check, no treatment	....	....	....
XVI *A*	(1/3 acre) 3 Klondike	{Diatomaceous earth White oil, 80 sec. visc.	94 lbs.} 6 pts.}	Dust	3
B	2 Klondike	Check, no treatment	....	....	....
XVII	(10 acres) (Soil moisture control)	*No insecticidal treatment given*			
A[o]	36 Eureka + 13 Neff	Moisture deficiency from Aug. 20 to end of season		....	....
B	42 Eureka + 7 Neff			....	....
C	49 Eureka	Ample supply moisture throughout entire season		....	....
D	49 Eureka			....	....
	Totals—59 plots, 2,160 walnut trees, 1,048 peach trees, 125 acres			....	....

[a] See pp. 529–530 for method of determining population rating.

[b] Data based on count of nuts on trees just prior to harvest (September 28).

[f] These were trees surrounding experimental plots for purpose of isolation, called "contact trees" in text.

[j] Actual increase in degree of infestation over check.

[m] Estimated average for all Eureka and Franquette trees in grove.

[n] Estimated average for all Klondike trees in grove.

[o] Below wilting point during late July.

TABLE 31—*(Concluded)*

Number of applications	Date	Foliage injury "burn"	Average infestation, 1930		Population rating[a]	Results[b]			Per cent control
			Nuts per tree	Per cent		Trees counted	Total nuts counted	Mean per cent infested	
2	7/9, 8/1	None			1	20	4,824	13.9±1.6	15.3±21.9
2	7/9, 8/1	Slight	....	10[m]	1	14	2,994	3.5±0.7	78.7± 8.5
....	....	....			2	19	3,016	16.4±3.5	....
2	7/15, 8/10	None	9	....	2	16	1,855	11.1±1.3	40.0± 7.6
1	7/13	None	9	....	1	16	1,493	24.5±1.8	+30.0±15.1[j]
1	7/13	Slight	22	....	2	15	3,025	15.1±1.2	18.4± 7.6
1	7/12	Slight	8	....	1	4	832	4.4±0.7	76.3± 7.6
....	....	....	11	....	2	15	2,779	18.5±1.6	....
1	7/15	None		10[n]	1	3	1,050	2.8±1.5	10.0± 0.7
....	....	....	....		1	2	837	3.1±0.7	....
....	....	....	....	2	3+[p]	4[q]	6,745	44.5±2.1	
						36[r]	55,271	49.0	45[s]
....	....	....	....	2	3+[p]	4[q]	6,213	60.2±9.0	
						42[r]	63,441	54.2	
....	....	....	....	6	3+[p]	4[q]	2,745	89.9±0.9	
						49[r]	37,895	95.1	....
....	....	....	....	1	3+[p]	48[r]	23,636	96.4	
....	....	....	....	....	....	999	333,707	....	....

[p] Approximately 1400 flies colonized.

[q] Key trees.

[r] All trees in plot.

[s] Indicates effectiveness of soil moisture deficiency. Based on average of percentage infested on "key trees" plus "all trees" in plots *A* and *B* as compared with plots *C* and *D*.

[t] 2 per cent nicotine content.

Plot Experiment I.—Plots in this experiment total approximately 45 acres, which constitutes a portion of a 100-acre Eureka planting. Mature peach trees are interplanted between walnut trees in the rows from
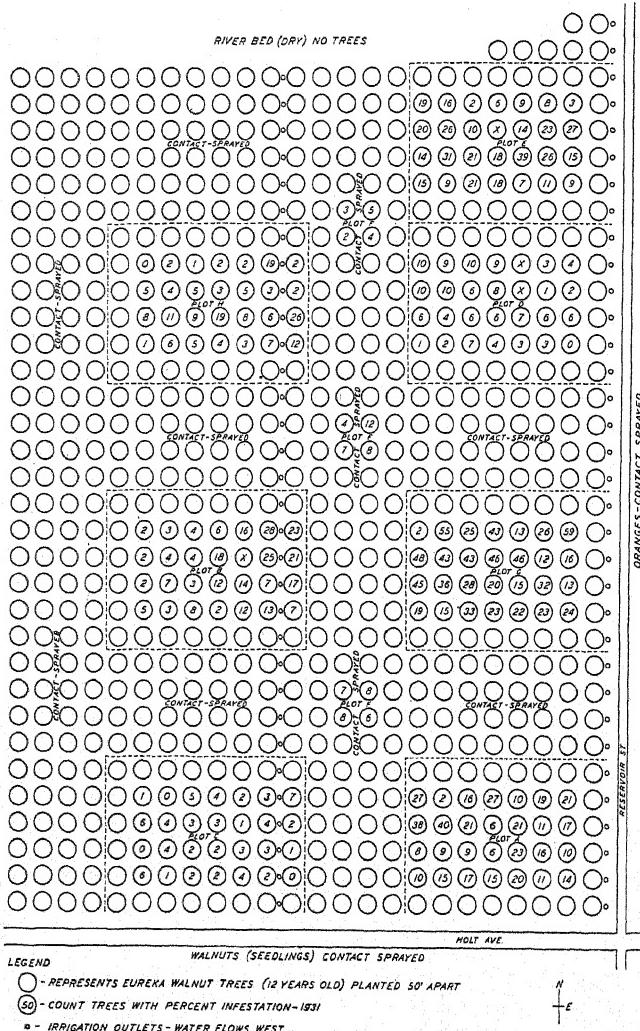
Fig. 72. Layout of plots in experiment I (45 acres);
1931 *Rhagoletis completa* control studies.

east to west. With the exception of plot E all plots were isolated in the same manner as in 1930. Figure 72 illustrates the arrangement of plots. Since the northern limits of the planting had been reached, it was not possible to maintain plots comparable in size and yet to isolate plot E

from plot D by the customary 4-row contact area. However, since the direction of the prevailing wind is from the northwest, and all trees in plot D as well as in the contact area to the west were sprayed, it is believed that there was not an appreciable drift of flies into plot E. In all instances, except in plot E, the peach interplants received the same treatment as the walnut trees.

Two dust applications of barium fluosilicate (plot C) afforded a fairly satisfactory degree of control. One dust application was approximately one-half as efficacious as two dust applications. The incorporation of 2 per cent fish oil in the dust mixture (plot B) materially increased the efficiency of one dust application (plot A). One spray application (plot D) resulted in a considerably higher degree of control than one dust treatment (plot A). In plot E, where the interplanted peach trees were not treated, the degree of control obtained was approximately one-half of that of plot D, in which the interplants were treated.

The results of one spray application in the contact area (plot F) were equivalent to those obtained in plot D, which received the same treatment. Only one tree separated the count trees of the contact areas from the nearest corner tree of a plot; the similarity of results in plots D and F might indicate that adequate isolation was provided under these conditions. However, it is doubtful if a buffer zone one tree deep between plots would preclude fairly free movement of flies from one plot to another. The method used in this experiment for plot isolation and the number and location of count trees to determine infestation were entirely satisfactory. Figure 72 illustrates the location of count trees in various plots and also furnishes a record of the percentage of infestation on individual trees.

The relation of fly population to walnut population, and the resulting percentage of infestation, is a matter of interest and one that requires thorough study. In plot H in 1930 there was an average of one infested nut per tree; while in 1931 there were approximately 25 infested nuts per tree. In plot G in 1930 the average of infested nuts per tree was 9; while in 1931 it increased to 78. The ratio of increase in infestation to expected female population in the two plots is not equivalent. Such factors as these are constantly operating in field control experiments; until detailed information regarding their nature is available, accurate comparisons of treated and untreated plots cannot be made.

Plot Experiment II.—In this grove, trees of the Placentia variety were planted alternately with Eurekas. The Placentia is very resistant to attacks by the fly, while the Eureka is very susceptible. This experiment was one of several intended to determine whether treating all trees within a solid block is necessary in order to obtain best results. The trees

were not very large; therefore 3 pounds of material per tree was a relatively heavy application. The data indicate that when all trees within a plot were treated, as good results were obtained from one application as were obtained from two applications. This evidence is contrary to that in experiment I. Where two applications were made (plot D) without treating the Placentia trees, as good results were obtained as in those plots where the Placentias were treated. Appreciable amounts of dust drifted into the adjoining trees, thereby enhancing the degree of control obtained for the plot. However, the results were poorest where one application was made without treating the Placentia trees. All treated plots had much lighter infestations than the untreated plots.

Plot Experiment III.—These tests were conducted in a grove of small Eureka trees that were interplanted with mature peach trees. They were designed to determine the value of treating peach interplants. The results do not show significant differences between the two treated plots, while the small percentage of infestation in both, when compared with the untreated plot, demonstrates the effectiveness of the material.

Plot Experiment IV.—This experiment was a direct comparison of one dust application of barium fluosilicate with one of basic lead arsenate. The data indicate that barium fluosilicate is superior to lead arsenate, even though the results in both instances were unsatisfactory.

Plot Experiment V.—In this experiment tests were conducted to determine whether or not the incorporation of an adhesive material in barium fluosilicate spray would result in increased effectiveness. Therefore light-pressed fish oil was included in the spray mixture. In comparing the degree of control obtained in this test with that of comparable plots similarly treated but without fish oil, no significant difference is apparent. The remaining plot in this experiment was dusted with hydrated lime. Results indicate that this material possesses considerable merit, thus warranting further trials. The nature of the action of this nonpoisonous substance is not understood.

Plot Experiment VI.—This is one of several experiments designed to determine how extensive an area surrounding individual trees of susceptible varieties must be treated in order to accomplish satisfactory control. The Eureka trees in this grove were interspersed singly among trees of the Placentia variety. Plot A consisted of three Eureka trees in different locations in the Placentia grove. These three trees were sprayed, while surrounding Placentia trees remained untreated. In plot B each Eureka tree was sprayed, together with a barrier zone of Placentia trees one tree deep, completely encircling the Eurekas. The data indicate unsatisfactory results in both plots, suggesting that the treated

barrier zone was not extensive enough. However, the infestation was lower in degree where the treated barrier zone was maintained.

Plot Experiment VII.—The purpose and layout of this experiment were similar to those of experiment VI but with synthetic cryolite in place of barium fluosilicate. The data corroborate experiment VI, in that a treated barrier zone one tree deep encircling individual susceptible trees is not extensive enough to give satisfactory results. If, as seems questionable, comparisons may be made between plots in different orchards, experiments VI and VII indicate that synthetic cryolite is superior to barium fluosilicate in effecting control of the fly.

Plot Experiment VIII.—This experiment was similar in purpose and layout to experiments VI and VII, but with a dust of natural cryolite as the treatment. The data indicate insignificant differences in the percentage of control obtained in treated and untreated plots.

Plot Experiment IX.—Plots A and B in this experiment afford a comparison of the effectiveness of natural cryolite with that of the synthetic product. Two dust applications of the natural product were more effective than one dust application of the synthetic material. However, the performance of one dust application of synthetic cryolite was considerably more effective than one dust application of barium fluosilicate in plot A of experiment I. The relative sizes of the trees in the two plots were such that the amounts of material applied per tree were comparable. Observations showed that synthetic cryolite adhered to the foliage better than did barium fluosilicate; this fact may partially explain any differences in percentage of control obtained with the two materials. The data furnished by plot C indicate that talc is apparently of no value in effecting control of the fly. Results in the contact area compared with those of contact areas in other experiments suggest that flies migrated into this area from plots C and E. The fly population in these two plots was relatively dense throughout the season.

Plot Experiment X.—This experiment did not yield any information regarding the efficacy of synthetic cryolite. The grove was heavily infested in 1930, but in 1931 only a very small percentage of flies emerged. Furthermore, the walnuts apparently were not in a susceptible condition during the activity of those flies that emerged.

Plot Experiment XI.—The efficacy of nicotine as a stomach poison in the control of the walnut husk fly warranted investigation. Experiment XI was one of several conducted to obtain information regarding this matter. Smith[35] determined that nicotine sulfate may be placed in solution in oil through the use of butanol as an intermediate solvent. The volatility of nicotine from a solution of this nature is greatly decreased and, therefore, when applied to walnut foliage the nicotine

offered a possibility of action as a stomach poison. However, the results of this test indicate that the material has no value in controlling the fly. A moderate degree of foliage injury was evident on all trees treated.

Plot Experiment XII.—The promising use of nicotine tannate in the control of the codling moth, *Carpocapsa pomonella* (Linn.)[21] suggested this test of the value of nicotine in the control of the walnut husk fly. Consequently two applications were made in plot A in comparison with two applications of barium fluosilicate in plot B. The size of the planting did not permit the usual isolation of plots. Each plot was only four trees wide, one adjoining the other. The results are not very significant, as evidenced by the percentage of control in the plots treated with barium fluosilicate when compared with the performance of this material in other experiments where only one spray application was made.

Plot Experiment XIII.—In this experiment the efficacy of nicotine sulfate plus sucrose as spray was compared with nicotine sulfate plus bentonite as a dust. In both mixtures the volatilization of nicotine was greatly retarded. The results indicate approximately equal degrees of control for these two materials. In both instances control was unsatisfactory.

Plot Experiment XIV.—When nicotine sulfate is incorporated with diatomaceous earth the volatilization of nicotine takes place very slowly. Therefore this mixture may be expected to possess value as a stomach poison. Experiment XIV served to compare the efficacy of nicotine sulfate plus diatomaceous earth as a dust with that of barium fluosilicate plus talc as a dust. The results indicate insignificant differences in percentage of infestation in the plot treated with nicotine and earth and the untreated check plot. The performance of barium fluosilicate plus talc was equivalent to that obtained in similarly treated plots in other experiments.

Plot Experiment XV.—This experiment was designed to determine the merits of finely ground tobacco dust containing 2 per cent nicotine, of diatomaceous earth, and of barium fluosilicate undiluted. The results indicate considerable value for tobacco dust, and none for diatomaceous earth. The results from a single application of straight barium fluosilicate are inconclusive, in view of the fact that there were more than twice as many infested nuts per tree in 1930 in this plot as in any other. A heavier infestation developed in treated plot B than in the untreated check plot. This illustrates the nature of some of the uncontrollable variables in experiments of this type.

Plot Experiment XVI.—In this experiment the diatomaceous earth and mineral oil dust was shown to be valueless in the control of the fly.

Plot Experiment XVII.—This was an experiment in applied ecology, in which an effort was made to manipulate the environment in such a manner as to render the walnuts resistant to oviposition attacks by the fly. The available information concerning irrigation and susceptibility
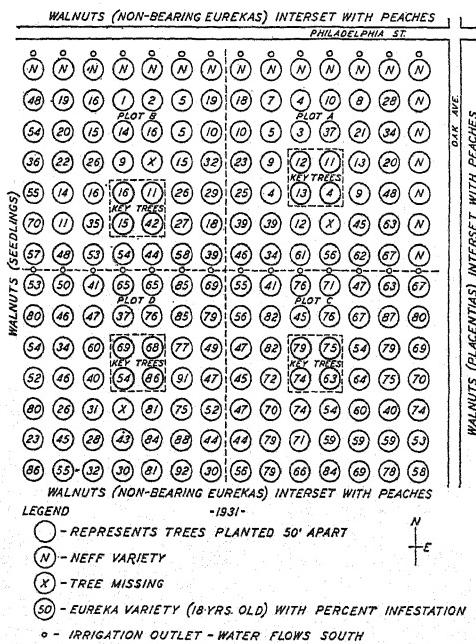

Fig. 73. Layout of plots in experiment XVII, on differential irrigation practice (10 acres), 1931 *Rhagoletis completa* control studies.

to infestation encouraged further investigation. Therefore the purpose of experiment XVII was to determine whether or not a relation exists between the amount of soil moisture present and susceptibility to attack by the fly and also to determine whether the degree of infestation can be influenced by manipulation of soil moisture. The portion of the experiment dealing with soil moisture was conducted in coöperation with O. L. Braucher.[5]

Four plots were laid out in a 16-year-old, 10-acre Eureka grove (fig. 73). A detailed history of the walnut-husk-fly infestation in this grove was available. The minimum number of trees in any plot was 36. Four

[5] Assistant in Orchard Management, University of California Citrus Experiment Station, and Field Investigator, California Walnut Growers' Association.

adjoining trees in the approximate center of each plot were selected for detailed observations and are referred to as "key trees" in the ensuing discussion.

The soil in plot A was permitted to go far below the "wilting point" during the latter part of July, which fact constituted the chief difference in soil-moisture manipulation between plots A and B. Both plots were subjected to a deficiency of soil moisture from the middle of August until after the crop was harvested. Therefore the moisture control exercised in these two plots was similar enough to warrant consideration of them as duplicates in this respect. Plots C and D were irrigated frequently enough to maintain soil moisture very close to the field capacity at all times. For this reason these two plots are considered as duplicates.

The fly population on the key trees was augmented in the early portion of the season by daily liberation of flies that were collected in the emergence cages. Equal numbers were liberated each day until a total of 348 flies had been colonized per key tree. From detailed studies of this grove during 1930, together with other data pertaining to pupal mortality, adult emergence, and percentage infestation, the approximate total number of flies present per tree was calculated. The average number of female flies per key tree, and the average number per tree in each plot (calculated from natural population, plus colonization) was as follows:

Plot	Flies per key tree	Flies, per tree, entire plot
A	196	41
B	211	54
C	306	146
D	199	40

Soil samples were taken at 10-day intervals, or more frequently when necessary, in the areas occupied by the key trees, and the moisture content was determined. Likewise at frequent intervals a composite sample of 50 walnuts was taken from the trees immediately surrounding the key trees in each plot, and the hardness of the husk was determined. These husk-hardness data are presented in figures 74, 75, and 76. This grove did not receive any insecticidal treatment for walnut-husk-fly control during 1931.

A double check on the degree of infestation was made. The first was a tree count prior to the beginning of harvest, to determine the percentage infestation at that time. These data for each tree are shown in figure 73. The second check was the harvest record in which the data for each key tree were kept separate; however, the data for the remainder of the trees in each plot were collected as a unit. A summary of certain of the

data obtained from this experiment, including the harvest record, is presented in table 31.

The data indicate a reduction in infestation of 45 per cent in plots A and B, when compared with plots C and D (table 31). There was a total of 118,712 nuts or possible host sites in the combined plots A and B and a calculated total of 3,744 females, or an average of 31.7 nuts per female. In the combined plots C and D there was a total of 61,531 nuts and a calculated total of 9,074 females, or an average of 6.8 nuts per female. In view of these data, the differences in percentage infestation between plots A and B, and C and D, as shown in table 31, cannot be considered significant. Therefore moisture control as practiced in these plots is apparently of negligible value in reducing infestation by the fly. Furthermore Braucher[7] concluded from his detailed study of soil variation within the confines of this 10-acre grove, together with the soil-moisture data, that the margin of safety to the general health of the tree and to the quality of the developing crop is not sufficient to warrant withholding moisture from the trees at such a critical time.

In comparing percentages of infestation from tree counts as shown in figure 73 with those for the harvest record which are given in table 31, certain features require clarification. The tree count to determine percentage infestation was made on September 26. At this time the husks of earlier-ripening walnuts were beginning to split. A tree count made 7 to 10 days later would not have yielded satisfactory data, since the husk of the uninfested walnut frequently darkens on the inside shortly after splitting, thereby rendering it somewhat similar in appearance to an infested walnut when viewed from a distance. A period of approximately 30 days usually elapses from the time the husks begin to split until the harvest is completed. Since characteristic husk blackness is the most feasible symptom to use in determining infestation from tree counts, those late-infested walnuts are not detected. However, the ratio of tree counts to final infestation was found to be similar in all plots.

This experiment supplied certain information regarding dispersion of flies within a grove. Percentages of infestation, as indicated by tree counts, are shown by the numerals within the tree circles in figure 73. These data indicate that the flies liberated on the key trees dispersed in all directions in a fairly uniform manner and reached the boundaries of the grove, a distance of 150 feet from the nearest key tree. It is the general opinion among many workers dealing with fruit flies, and particularly with economically important *Rhagoletis*, that the flies remain fairly well localized unless conditions exist that are unfavorable to oviposition. It is of interest to note the heterogeneity in degree of infestation in this grove and particularly within any one plot.
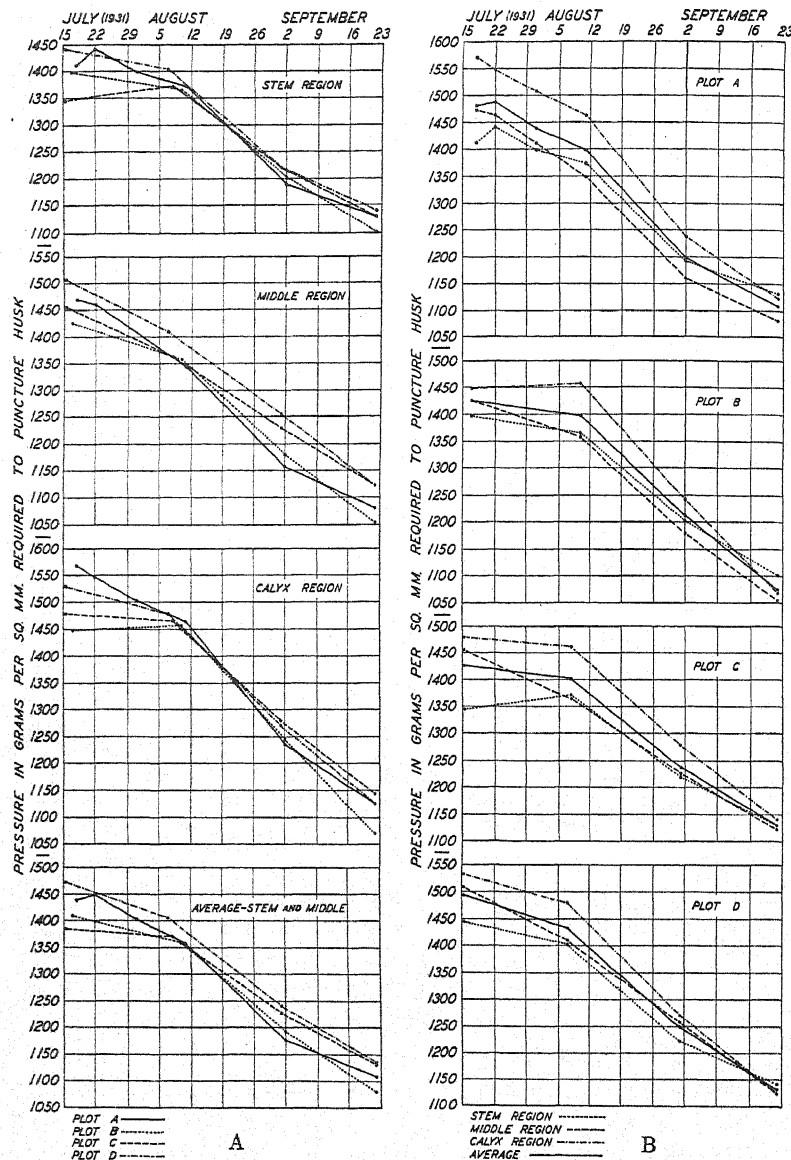
Fig. 74. Husk-hardness data regarding Eureka walnuts in experiment XVII, on differential irrigation practices, 1931 *Rhagoletis completa* control studies.

The husk-hardness data for the individual plots (fig. 74 *A*) show that in all instances the calyx region of the nut was hardest throughout most of the season of fly activity. There was no consistent significant difference in the hardness of the stem and middle regions in any of the four plots. A comparison of the husk hardness in the various regions in each of the four plots (fig. 74 *B*) does not present differences of sufficient
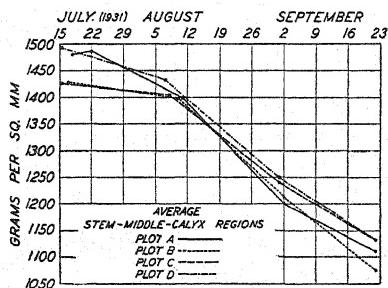

Fig. 75. Summary of husk-hardness data regarding Eureka walnuts in irrigation experiment XVII, on differential irrigation practices, 1931 *Rhagoletis completa* control studies.
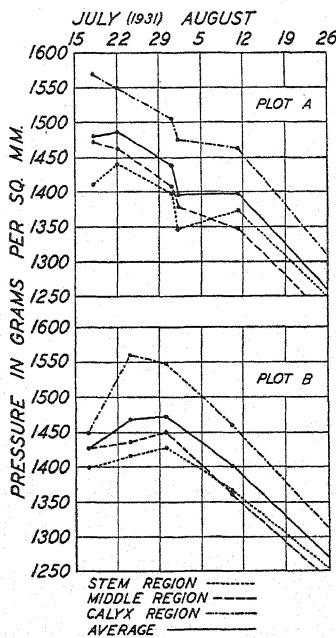

Fig. 76. Relation of irrigation to husk hardness in experiment XVII, on differential irrigation practices, 1931 *Rhagoletis completa* control studies. In plot A, the application of irrigation water on July 31 resulted in the husks' becoming softer immediately.

magnitude to be considered significant. The averages of the hardness of the three regions of the walnuts (fig. 75) in the four plots do not show significant differences between any of the plots.

Figure 76 shows that husk hardness is influenced to some extent by soil moisture under certain conditions. In plot A the soil moisture was well below the "wilting coefficient" on July 31. The average husk hardness of the three regions of walnuts at this time was approximately 1,445 grams per sq. mm. This plot was irrigated on July 31, August 1, and August 2, and the husk-hardness data were collected on August 2,

after irrigation was completed. At this time the average hardness of the three regions of the walnuts was approximately 1,395 grams per sq. mm. Therefore it is evident that the application of water to the soil was responsible for the husks' becoming 50 grams per sq. mm softer. Each region of the husk became softer, though the calyx region did not soften to the same degree as the stem and middle regions. Withholding moisture from the soil during July in plot A appears to have caused the husks to reach the peak of hardness by mid-July, at which time they began to soften; while in plot B, with normal irrigation during July, the husks reached the peak of hardness several weeks later.

Discussion of Field Control Experiments in 1931.—Seasonal conditions in 1931 were not considered entirely favorable for maximum economy in the biology of the walnut husk fly. Forty days intervened between the date when 50 per cent of the flies had emerged and that when the walnuts reached apparently optimum condition for oviposition. No doubt an appreciable number of flies died without depositing any eggs. Furthermore, the walnuts ripened approximately 10 days earlier than normally, thereby somewhat shortening the period for effective activity of the fly.

The size of the walnut crop was classified as "light." Therefore in most instances where ineffectual control was obtained, the degree of infestation was relatively heavy when expressed as a percentage.

The experimental plots supplied valuable information regarding the efficacy of various materials, the timing of treatments, and the method of application. In evaluating the results the probable error of the mean percentage of infested nuts is given, which indicates the significance of the data. The probable error of percentage of control in each instance indicates the reliability of the comparison of treated plots with untreated checks.

The following conclusions appear justifiable: Two dust applications of barium fluosilicate or cryolite, properly timed with respect to fly emergence, afforded satisfactory control. One dust application when the flies began to emerge was unsatisfactory; however, synthetic cryolite was apparently superior to barium fluosilicate under these conditions. One spray application of barium fluosilicate under these conditions was more effective than one dust application, though it did not afford satisfactory control. The incorporation of an adhesive material in the fluorine dusts is highly desirable. Of the various combinations employing nicotine as a stomach poison, nicotine tannate was the most effective; however, it was not so promising as either barium fluosilicate or cryolite.

All trees within a solid block must be treated to accomplish satisfactory results. Furthermore an area of trees or other vegetation adjoining susceptible varieties should be treated to insure maximum control efficiency. When a single tree or a few susceptible trees are growing interspersed with resistant varieties, treatment of a zone at least two trees deep is necessary for adequate protection. It is questionable whether or not the expense of this treatment would be justified by the increase in returns. Such a mixed planting condition is usually undesirable; therefore the removal of susceptible trees and replanting or topworking with resistant varieties is probably the best procedure. The available information regarding alteration of the environment through manipulation of soil moisture in an effort to inhibit oviposition activities of the fly is such that further experimentation is unwarranted.

Adhesives Incorporated in Dust Mixtures.—Fairly extensive field tests on both walnut and citrus trees have shown that in general the more commonly used fluorine compounds do not adhere well to foliage, but they adhere better when applied as spray than as dust. During August, 1931, an unseasonal rain of from 0.20 to 0.50 inch resulted in the loss of a large percentage of the barium fluosilicate and synthetic cryolite from the foliage of trees in dusted plots. Very appreciable amounts of those materials are lost from walnut foliage as a result of runoff in atmospheric dew. Since the cost of application of materials for walnut-husk-fly control favors the dust method, an effort was made to increase the adhesiveness of dust materials, particularly barium fluosilicate and synthetic cryolite. Hood[22] showed that the incorporation of fish oil in lead arsenate sprays greatly increased the period over which it adhered to the foliage of forest trees in the New England states. Marcovitch and Stanley[27] recommend fish oil in dusts of barium fluosilicate and cryolite used for the control of the Mexican bean beetle, *Epilachna corrupta* Muls., in Tennessee. Tests were first conducted here on citrus in 1929 using various percentages of a light-pressed herring oil and sperm oil incorporated in both barium fluosilicate and cryolite dust mixtures. Both talc and diatomaceous earth were used as diluents. Where 8 per cent of either of these oils was used, most of the material remained on the foliage through the winter rainy season.

For the tests with adhesive materials on walnuts during 1931, fish oil classified as light-pressed herring oil, raw linseed oil, cottonseed oil, and highly refined mineral oils of viscosities ranging from 60 to 100 seconds Saybolt were each used at concentrations by weight of 2, 4, and 8 per cent. Fiber talc and diatomaceous earth were each used as diluents with both barium fluosilicate and synthetic cryolite. In all instances where the oils were used at a concentration of 2 per cent the sticking

qualities of the dust were unsatisfactory; at 4 per cent the materials adhered fairly satisfactorily to the foliage. At a concentration of 8 per cent the adhesive qualities of the dust mixture were very good; however, where talc was used as the diluent the increased specific gravity of the mixture greatly reduced the mechanics of application of the dust. When diatomaceous earth was used as a diluent the incorporation of 8 per cent of any of the oils did not adversely affect the dusting qualities of the mixture; and in fact in one instance where 16 per cent of an 80-seconds-viscosity mineral oil was incorporated in the dust mixture of diatomaceous earth and barium fluosilicate, the dusting properties were not materially reduced. The experience gained in mixing these dust formulas corroborates the findings of Flint and Farrar,[14] that oil dusts cannot be satisfactorily mixed in the hopper of the regular self-mixing dusting machines. In the walnut-husk-fly control studies, the most satisfactory oil dusts were obtained where the oil was atomized into the toxic-diluent mixture in a special dust-mixing machine, and the final mixture broken up or fluffed by a rotary fiber brush revolving in a fine-mesh screen half-cylinder in the discharge mechanism of the mixer.

The field tests as conducted indicated the superiority of drying oil to mineral oil, at comparable concentrations, for sticking dust particles to foliage. The drying oil was completely dry within 2 to 3 days after application.

Diatomaceous earth was somewhat superior to other diluent materials tested, for it was not only especially light before the incorporation of oil, but also its dusting properties were not materially affected by the oil. However, in field application when mineral oils were incorporated as an adhesive agent in mixtures in which diatomaceous earth was the diluent, the rate of discharge of the dust could not be controlled as satisfactorily as when fish oil was used. Since the viscosity of the fish oil was nearly twice that of the heaviest mineral oils used, this factor may be related to the observed differences. When talc was used as the diluent, the rate of discharge could be controlled satisfactorily regardless of the type of oil that was incorporated for adhesive purposes.

Storage tests of fish-oil dusts show that the oil dries unless the air is excluded, thereby losing its effect as an adhesive. Furthermore, where higher percentages of fish oil (6 or 8 per cent) are used, particularly with talc as a diluent, if normal oxidation is permitted in storage, the dust mixture is likely to "heat up," thereby charring the material. Therefore the most satisfactory procedure, when practical, is to apply the fish oil-dust mixture soon after it has been mixed.

For the control of insects possessing sponging and sucking mouth parts, the probable performance of insecticide dusts in which oil had

been incorporated is a matter of conjecture. The use of dusts in which a sufficient amount of fish oil is included to insure maximum adhesiveness results in the particles' adhering very firmly to the leaf tissue; and the insect cannot "pick up" the particles of material as readily as when no adhesive material is used. When mineral oil is used in dust mixtures for adhesive purposes, the insecticide particles do not adhere to the leaf as firmly as when drying oil is used. On this basis, the mineral oil appears more promising than fish oil as an adhesive in dust mixtures for the control of the walnut husk fly.

Ferric oxide was used for adhesive purposes in several tests at concentrations of 20 and 50 per cent in the barium fluosilicate and talc mixture. The ferric oxide adherred to the foliage but there was apparently no appreciable increase in adherence of the insecticide.

FIELD INVESTIGATIONS IN 1932

In 1932 there were 23 field control plots comprising approximately 55 acres. The experimental work on control was mainly to determine the efficacy of: (1) 20 and 30 per cent cryolite-dust mixtures, with mineral oil and with fish oil as the adhesive, when one application was made just prior to the peak of emergence in contrast to two applications at the previously mentioned times; and (2) several materials that had not been previously used on walnuts or in husk-fly control experiments. Most of the plots were located in groves that were used for experimental purposes in 1931; therefore the detailed history of infestation was available. Unfortunately it was not feasible to maintain untreated controls in any of the experiments; furthermore, practically every grove was treated in which an appreciable infestation was known to exist. Therefore an accurate evaluation of the performance of the materials and treatments was not possible except in comparison with one another. The conduct of the experiments was very similar to that of 1931. In two groves, the previously described system, that is, spraying of contact areas, was carried out in isolated plots. In the other experimental groves the plots were sufficiently large to insure satisfactory isolation of their central areas, from which the data were taken.

Adult emergence began June 29 and the seasonal peak was reached August 10 (fig. 45, p. 417).

The data obtained from the field control plots in 1932 are summarized in table 32.

Plot Experiment I.—This experiment was primarily designed to compare the efficacy of synthetic cryolite (sodium fluoaluminate), potassium fluoaluminate, magnesium arsenate, and acid lead arsenate. Cryolite and potassium fluoaluminate are apparently more effective in pro-

TABLE 32

CONTROL EXPERIMENTS IN 1932

Experiment	Number of trees and variety	Material	Application					Total cost per acre of walnuts (17 trees)	Per cent infested 1931	Results[a]		
			Concentration	Method	Amount per tree	Number	Date			Trees counted	Total nuts counted	Mean per cent infested
I	(10 acres)											
A	25 Eureka	Cryolite[b] / Talc (fiber) / Mineral oil[c]	20 lbs. / 75 lbs. / 5 lbs.	Dust	3 lbs.	2	7/29, 8/31	$5.50	24.5	9	4,167	0.6±0.2
B	25 Eureka	Potassium fluoaluminate / Talc (fiber) / Mineral oil	20 lbs. / 75 lbs. / 5 lbs.	Dust	3 lbs.	2	7/29, 8/31	7.00	11.1	9	4,027	0.5±0.2
C	25 Eureka	Magnesium arsenate / Lime (hydrated) / Mineral oil	20 lbs. / 75 lbs. / 5 lbs.	Dust	3 lbs.	2	7/29, 8/31	9.00	18.5	9	6,007	1.9±0.3
D	25 Eureka	Acid lead arsenate[d] / Lime (hydrated) / Mineral oil	20 lbs. / 75 lbs. / 5 lbs.	Dust	3 lbs.	2	7/29, 8/31	6.00	15.1	9	6,107	2.9±0.3
E	96 Eureka[e]	Cryolite / Fish oil[f]	3 lbs. / 1 pt.–100 gals.	Spray	30 gals.	1	7/27	5.25	4.4	9	4,409	0.5±0.2
F	14 Eureka[g] { 7 trees treated as in Experiment C / 7 trees treated as in Experiment D			Dust	1½ lbs.	2	7/29, 8/31	.....	16.8	9	6,686	9.2±1.4
G	1 Eureka[h]	Check, no treatment							Approx. 15.0	1	435	21.8

[a] Data based on count of nuts on trees just prior to harvest (September 26).

[b] Synthetic cryolite used in all experiments.

[c] Mineral oil specifications: 95 sec. visc., 92 per cent unsulfonatable residue.

[d] Moderate foliage burn.

[e] These were trees surrounding experimental plots for purpose of isolation; called "contact trees" in the text.

[f] Fish oil classified as: light-pressed herring oil.

[g] Border row adjoining untreated grove; trees treated from one side only.

[h] Single tree in Placentia grove; 50 feet distant from Plot F.

TABLE 32—(Continued)

Experiment	Number of trees and variety	Material	Concentration	Application				Total cost per acre of walnuts (17 trees)	Per cent infested 1931	Results[a]		
				Method	Amount per tree	Number	Date			Trees counted	Total nuts counted	Mean per cent infested
II	(10 acres)											
A	25 Eureka	Cryolite 20 lbs. Diatomaceous earth 75 lbs. Mineral oil 5 lbs.		Dust	3 lbs.	2	7/29, 8/29	$5.50	96.4	9	4,664	1.1±0.2
B	25 Eureka	Cryolite 20 lbs. Diatomaceous earth 75 lbs. Fish oil 5 lbs.		Dust	3 lbs.	2	7/29, 8/29	5.50	54.2	9	6,523	0.4±0.2
C	25 Eureka	Cryolite 30 lbs. Diatomaceous earth 65 lbs. Mineral oil 5 lbs.		Dust	3 lbs.	2	7/29, 8/29	6.00	95.1	9	5,872	0.5±0.2
D	25 Eureka	Cryolite 30 lbs. Diatomaceous earth 65 lbs. Fish oil 5 lbs.		Dust	3 lbs.	2	7/29, 8/29	6.00	49.0	9	7,045	0.2±0.2
E	96 Eureka[c]	Cryolite 3 lbs. Fish oil 1 pt. 100 gals.		Spray	35 gals.	1	7/27	6.00	Approx. 75.0	9	8,761	0.8±0.2

[a] Data based on count of nuts on trees just prior to harvest (September 26).

[c] These were trees surrounding experimental plots for purpose of isolation; called "contact trees" in the text.

TABLE 32—(Continued)

Experiment	Number of trees and variety	Material	Concentration	Application Method	Amount per tree	Number	Date	Total cost per acre of walnuts (17 trees)	Per cent infested 1931	Results Trees counted	Total nuts counted	Mean per cent infested[a]
III	(15 acres) Peach interplants											
A	64 Eureka + 64 peach	Cryolite / Diatomaceous earth / Mineral oil	20 lbs. / 75 lbs. / 5 lbs.	Dust	{W^i 2½ lbs. / P^i ½ lb.}	1	8/11	$2.75	6.2	12	7,669	0.4± 0.2
B	64 Eureka + 64 peach	Cryolite / Diatomaceous earth / Mineral oil	20 lbs. / 75 lbs. / 5 lbs.	Dust	{W 2½ lbs. / P ½ lb.}	2	7/28, 8/29	5.50	17.1	12	8,150	0.4± 0.2
C	64 Eureka + 64 peach	Cryolite / Diatomaceous earth / Mineral oil	30 lbs. / 65 lbs. / 5 lbs.	Dust	{W 2½ lbs. / P ½ lb.}	1	8/11	3.00	16.9	12	6,849	1.0± 0.2
D	64 Eureka + 64 peach	Cryolite / Diatomaceous earth / Mineral oil	30 lbs. / 65 lbs. / 5 lbs.	Dust	{W 2½ lbs. / P ½ lb.}	2	7/28, 8/29	6.00	30.7	12	7,179	0.6± 0.2
IV	(10 acres)											
A	61 Eureka + 61 Neff	Cryolite / Diatomaceous earth / Mineral oil	30 lbs. / 65 lbs. / 5 lbs.	Dust	3 lbs.	1	8/12	$3.00	12.8	16	9,021	2.8± 0.3
B	61 Eureka + 61 Neff	Cryolite / Diatomaceous earth / Mineral oil	30 lbs. / 65 lbs. / 5 lbs.	Dust	3 lbs.	2	7/29, 8/27	6.00	14.5	16	9,661	1.0±0.2

[a] Data based on count of nuts on trees just prior to harvest (September 26).
[i] W=walnut; P=peach.

TABLE 32—(*Concluded*)

Experiment	Number of trees and variety	Application								Results[a]		
		Material	Concentration	Method	Amount per tree	Number	Date	Total cost per acre of walnuts (17 trees)	Per cent infested 1931	Trees counted	Total nuts counted	Mean per cent infested
V	(9 acres)											
A	10 Eureka[j]	Cryolite / Diatomaceous earth / Mineral oil	30 lbs. / 65 lbs. / 5 lbs.	Dust	3 lbs.	2	7/28, 8/28	$6.00	Approx. 95.0	10	7,800	22.6± 2.2
B	10 Eureka[k]	Cryolite / Diatomaceous earth / Mineral oil	30 lbs. / 65 lbs. / 5 lbs.	Dust	3 lbs.	2	7/28, 8/28	6.00	Approx. 95.0	10	7,893	7.5± 0.9
C	130 Eureka[l]	Cryolite / Diatomaceous earth / Mineral oil	30 lbs. / 65 lbs. / 5 lbs.	Dust	3 lbs.	2	7/28, 8/28	6.00	Approx. 95.0	10	7,782	5.9± 0.4
D	6 Eureka[m]	Cryolite / Diatomaceous earth / Mineral oil	30 lbs. / 65 lbs. / 5 lbs.	Dust	3 lbs.	2	7/28, 8/28	6.00	Approx. 95.0	6	3,516	20.9± 1.6
VI												
A	5 Eureka[n]	Check, no treatment				...		...	...	5	1,288	57.2±11.5
	Totals—23 plots, 1054 walnut trees, 256 peach trees, 54 acres							...	...	221	142,171	...

[a] Data based on count of nuts on trees just prior to harvest (September 26).

[j] West border row adjoining untreated Placentia grove 50 feet distant.

[k] Row adjoining plot A, 50 feet distant.

[l] Major portion of grove, exclusive of outer two border rows on east and west.

[m] East border row adjoining untreated Placentia grove.

[n] Untreated isolated susceptible trees in adjoining groves of resistant varieties.

ducing fly mortality than the two arsenicals tested. The data indicate that magnesium arsenate is more effective than acid lead arsenate; however, the difference is not great enough to be conclusive. Magnesium arsenate had not been used previously in these control studies; therefore the effect upon the tree was carefully observed. No injury resulted in this plot; however, in the acid lead arsenate plot moderate foliage burn was evident. This corroborates previous work with this arsenical, thus eliminating it from further consideration in this project.

One spray application of cryolite (plot E) is evidently as effective as two dust applications. (Other experiments in 1932 indicate that there is little difference in control between one and two applications of dust.)

The trees of plot F constituted a border row and were adjacent to an untreated Placentia grove at the regular planting distance of 50 feet. Furthermore this outer row of Eureka trees was only treated from one side, since the presence of a wire fence did not permit access to the other side. A strip of alfalfa was growing in the space between the Eureka row and the first Placentia row. The control was unsatisfactory, apparently either because the trees were sprayed only from one side or because the adjoining trees and alfalfa were not treated. Flies were commonly observed on the Placentia trees throughout the season.

Plot G consisted of a single untreated Eureka tree that was growing in the row of Placentia trees adjoining plot F. Flies became very abundant on this tree as the season progressed. While this single-tree plot does not constitute a representative control, the data, together with the data from plot F, are strongly indicative of what would probably have occurred in the other plots had not action of the applied materials been effective.

Plot Experiment II.—The cost of cryolite control warranted investigation of the comparative efficacy of 20 per cent and 30 per cent cryolite dust mixtures, each with fish oil and mineral oil incorporated. Accordingly plots were treated in a manner designed to show the merits of each combination.

Fourteen emergence cages were located in this grove and a daily record of fly emergence was kept. As a result of the relatively heavy infestation here in 1931, enormous numbers of flies emerged during the 1932 season.

Data regarding the hardness of walnut husks, collected from this grove at intervals throughout the season, showed that the walnuts were in susceptible condition for infestation throughout the period of adult emergence. Therefore it seems logical to conclude that the status of infestation in the grove at the end of the season was the direct result of the treatments given.

The very light infestation in all plots indicates that the materials used were highly efficacious in controlling the fly. The existing differences in degree of infestation in the various plots are not of sufficient magnitude to warrant conclusions as to the comparative value of the individual combinations.

One application of cryolite as spray (plot E) is apparently as effective as two dust applications.

Plot Experiment III.—The 1931 experiments demonstrated that one dust treatment applied at the beginning of fly emergence produced unsatisfactory results. Yearly observations regarding the peak of oviposition have shown that most of the eggs are deposited during the latter half of August, regardless of when the peak of fly emergence is reached. Therefore it was important to compare the effectiveness of one treatment applied before August 15 with two treatments timed as recommended previously. Further information regarding the comparative values for 20 per cent and 30 per cent cryolite dust was also desirable.

The data of experiment III indicate an insignificant difference in efficacy between the two concentrations tested, and between one and two applications. Apparently one application of 20 per cent cryolite dust properly timed produces satisfactory results.

Plot Experiment IV.—This experiment was outlined to obtain further information regarding the efficacy of one and of two applications of 30 per cent cryolite dust timed as described in experiment III. The data indicate a difference of approximately 2 per cent in degree of infestation in favor of two applications, but this difference does not appear to justify the extra expense of two treatments.

Plot Experiment V.—This experiment furnished additional information regarding the value of treating a bordering zone of nonsusceptible trees in order to obtain satisfactory control. The infestation in this grove in 1931 was uniformly heavy. Treatment in 1932 was according to recommendations. This Eureka grove was bounded by untreated Placentia groves on the east and west, by a paved street on the north, and by a grove of small Payne trees, which were treated, on the south. This Eureka grove, and the Placentia groves adjacent to the east and west, were very similar in size of trees and in soil and tree management. Flies were commonly observed throughout the season on the adjacent west row of Placentia trees. Figure 77 shows the location of the several plots.

In plot A, the west border row of Eureka trees adjoining the untreated Placentia grove, a 23 per cent infestation developed, while in plot B (which was the next row east) the infestation was 8 per cent, and in plot C, in the center of the grove, 6 per cent. In plot D, the east border

row adjacent to the untreated Placentia grove, the infestation was 21 per cent.

The data demonstrate conclusively that failure to treat an adjoining zone of at least one row of resistant trees materially affected the degree of control obtained in the border row of this Eureka grove.
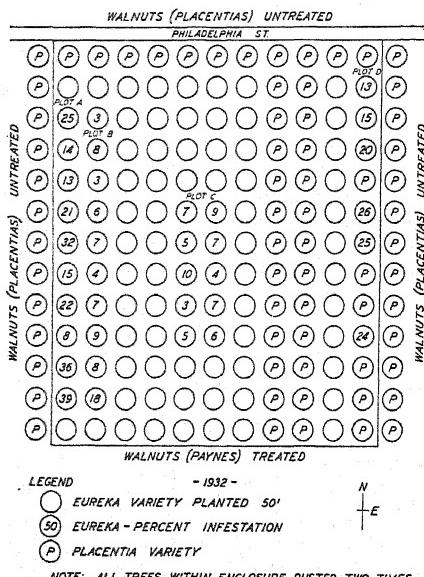
Fig. 77. Layout of plots in experiment V (9 acres), which demonstrates the necessity of proper isolation through treated barrier zones; 1932 *Rhagoletis completa* control studies.

Plot Experiment VI.—Observations were made on a few untreated Eureka trees singly isolated in groves of nonsusceptible varieties from various locations in the infested area. The infestation varied from 22 per cent to 99 per cent. Definite information regarding the degree of infestation in 1931 is lacking; however, the trees were reported to have been moderately to heavily infested. While these data cannot be considered as a control, they are indicative of the potential infestation in the experimental plots, which probably would have developed had no control measures been applied.

Discussion of Field Control Experiments in 1932.—The size of the 1932 crop of walnuts was considered large; therefore the performance of materials expressed in terms of percentage infestation is not directly comparable to that of the 1931 season, when the crop was small. How-

ever, size of crop in the experimental groves, with the exception of experiment V in 1931, was sufficiently uniform both seasons to warrant relative comparison of results in the various plots. The size of crop in experiment V in 1931 was larger than average for that year; thus with the high percentage (95 per cent) infestation the fly population in 1932 was considerably larger than in other groves.

Several important factors were responsible for mitigating the degree of infestation this season. Emergence records show that only approximately 67 per cent of the 1931 pupae produced annual-generation flies. Similar limiting effects were also operative on the emergence of biennial-generation flies. The walnuts ripened fully three weeks earlier than normal, thereby shortening the oviposition period somewhat. Furthermore, an appreciable percentage of those nuts in which oviposition took place after September 15 were not injured nor did the larvae mature. Data collected during harvest indicate that approximately 25 per cent of the infested walnuts ripened before the larvae attained sufficient size to cause the husks to blacken as a result of their feeding. Thus the degree of actual damage was appreciably lessened. To partially compensate for these factors adverse to the insect, the walnut husks reached the susceptible stage for oviposition at a more nearly optimum time for the fly with respect to the seasonal peak of emergence. The field-count method evidently indicated that under the circumstances existing this season, the mean percentage infestation more closely approached the degree of actual injury than it did in 1931. The data are believed to be as nearly comparable as conditions permit with this type of field-plot experimentation.

From the foregoing facts and in consideration of the data obtained from the field control plots, it seems logical to conclude that: One properly timed treatment of synthetic cryolite applied as dust or spray will afford satisfactory control. Two dust treatments of either potassium fluoaluminate or magnesium arsenate are also highly efficacious. Acid lead arsenate is an effective material but is unsafe to use from the point of view of tree health. Mineral oil and fish oil are apparently of equal value as an adhesive in the dust mixtures used. To insure satisfactory protection of susceptible varieties through insecticidal treatment, a zone of adjacent vegetation from 50 to 100 feet wide must be similarly treated.

RECOMMENDATIONS TO GROWERS ON CONTROL OF THE WALNUT HUSK FLY

On the basis of field control experiments, either synthetic cryolite[6] or barium fluosilicate is recommended for general use in the control campaign in the infested area. Synthetic cryolite from different manufacturers has been found to vary in the content of sodium fluoaluminate, uncombined sodium fluoride, and various sulfates, and also in solubility in water and such physical properties as fineness and bulk or weight. Therefore the following tentative specifications are suggested:

> Sodium fluoaluminate (Na_3AlF_6): not less than 97 per cent
> No uncombined sodium fluoride
> Free of sulfates
> Solubility: not more than 1 gram in 1,600 cc water at 20° C
> Mesh: 100 per cent through 200 mesh per inch; not less than 75 per cent
> 　　　through 325 mesh per inch (U.S. Bur. Standards Sieve Series No. 325)
> Volume (screened loose): not less than 48 cubic inches per pound

An adhesive is necessary in both sprays and dusts when either cryolite or barium fluosilicate is used. After preliminary experiments with various types of fish oils and vegetable oils, and with highly refined mineral oils of different viscosities, a mineral oil of the following specification appears most promising as an adhesive:

> Viscosity: 95 seconds Saybolt
> Sulfonation: 90 per cent

As a spray the recommended formula is:

> Synthetic cryolite or barium fluosilicate.................................... 3 pounds
> Mineral oil .... 1 pint
> Water ....100 gallons

From 30 to 40 gallons per average-sized tree affords satisfactory coverage.

The dust method of treatment is less expensive than the spray method. The recommended formula is:

> Synthetic cryolite or barium fluosilicate.................................30 per cent
> Diatomaceous earth ....65 per cent
> Mineral oil .... 5 per cent

From 3 to 4 pounds per average-sized tree affords satisfactory coverage.

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[6] Towards the termination of this project and afterwards, the preparation of natural cryolite for insecticidal purposes has been greatly improved and field trials elsewhere on other insects have demonstrated its efficacy in comparison with other fluorine compounds. Therefore it appears that this material may also be satisfactorily employed in the control of the walnut husk fly.

The proper diluent material for the dust mixture is of considerable importance. Of the many noncalcium materials tested, a special grade of diatomaceous earth and fiber talc appears most promising. Where an adhesive consisting of either mineral oil or fish oil is required in quantities of from 5 to 10 per cent, the diatomaceous earth is most satisfactory. Without an adhesive, fiber talc is entirely satisfactory. Since in this instance an oil adhesive is required, diatomaceous earth of the following tentative specifications is suggested:

> Silica as diatomes: not less than 90 per cent
> Free of calcium
> Moisture: not more than 5 per cent
> Mesh: 100 per cent through 200 mesh per inch; not less than 75 per cent
>   through 325 mesh per inch (U.S. Bur. Standards Sieve Series No. 325)
> Volume (screened loose): not more than 190 cubic inches per pound.

When this type of diluent is used, the bulkiness of the mixed dust possesses certain advantages, particularly from the point of view of application. The operator may permit a large volume of dust to discharge continuously while treating an individual tree and not be so likely to apply more than the relatively small dosage of 3 pounds specified. Talc is relatively heavy and therefore the operator must regulate the discharge carefully in order to prevent the application of a greater number of pounds per tree than is required. Because of weather conditions, practically all dust treatment is applied during the night; therefore the application is likely to be more uniform with a bulky dusting material. The disadvantages of this type of material are that the mixing costs are increased slightly, more sacks or containers are required, and stops for filling the dusting machine must be more frequent.

Proper mixing of the ingredients in the dust mixture is essential to insure best results. A modern machine designed for mixing of dust insecticides should be employed. Analyses have shown that a minimum time of 5 minutes is necessary to properly mix cryolite or barium fluosilicate and diatomaceous earth. After the oil is atomized into the fluoride-earth mixture, an additional 10 minutes' mixing is necessary to mix the oil thoroughly with the dust. Thus a total running time of 15 minutes per batch is required.

To insure adequate protection within a grove through insecticidal treatment, a zone of adjacent vegetation from 50 to 100 feet wide must be treated simultaneously with the infested grove. In view of the limited duration of the experimental work with these materials, two treatments are recommended, the first when adult emergence becomes regular, and the second approximately 4 weeks after the first. The 1932 data indicate that probably one properly timed application will afford satisfactory re-

sults. However, more extensive observations regarding seasonal history and seasonal host resistance are necessary before definite conclusions can be made regarding the most economical control measures.

SUMMARY

History.—*Rhagoletis completa* Cresson was introduced into California prior to 1926 from the central region of the United States (about the 100th meridian), where it is apparently indigenous. It was probably introduced as larvae or pupae in black walnuts. In 1927 the species assumed major economic importance as a pest of certain commercial varieties of Persian walnut, *Juglans regia*. The area of infestation has increased yearly, and in 1932 it comprised approximately 500 square miles and included over 2,000 acres of commercial varieties of Persian walnut. Taxonomists confused this insect with *R. juglandis,* and it was not until 1929 that it was found to be undescribed. Cresson then considered it a subspecies of *R. suavis.*

Taxonomy and Technical Description of Stages.—Studies dealing with large series of specimens of *Rhagoletis suavis* and its subspecies *completa* from different areas resulted in the elevation of *completa* to specific rank. Characteristic differences are evident in the pattern of the infuscated areas of the wing and in male genitalia. *R. completa* is of a general tawny color with yellowish-white markings. The wings are hyaline with three parallel transverse infuscated bands. The distal band continues along the anterior margin of the wing to the apex. Length, 4 to 8 mm.

The egg of *Rhagoletis completa* is somewhat curved in shape and pearly white in color. First-instar larvae are semitransparent, and are characterized by the absence of anterior spiracles, the presence of two peritremes on each posterior spiracle, and a tooth on the blade of each oral hook. Second-instar larvae possess anterior spiracles and three peritremes on each posterior spiracle. Mature third-instar larvae are creamy white. The tooth on the oral hook is absent. There are 14 tubercles situated on the posterior body segment. These structures, together with the angle that the lower peritreme makes with the horizontal, serve to identify the species. The pupa is somewhat barrel-shaped and of straw color.

Related Species Attacking Walnuts.—*Rhagoletis suavis,* the walnut husk maggot, occurs throughout most of the eastern United States on the black walnut, *Juglans nigra.* This species is reported to be economically important as a pest of Persian walnut in New York, Pennsylvania, and Maryland. *R. juglandis* is probably a Mexican species and is recorded from southern Arizona and Chihuahua, Mexico. Serious infesta-

tions have been observed on Persian walnut in Arizona, where it also attacks the native black walnut, *J. rupestris. R. boycei* is recorded from southern Arizona. It probably attacks wild and cultivated walnuts. Adults of these three species are illustrated.

Common Name.—"Walnut husk fly" was proposed as the common name for this insect in 1929, and was formally accepted in 1930. At that time the species was believed to be *Rhagoletis juglandis.* Since this common name was actually intended for this particular insect, it is retained, although *R. completa* is now known to be distinct from *R. juglandis.*

Distribution.—Authentic records show that *Rhagoletis completa* occurs in Nebraska, Kansas, Oklahoma, Texas, New Mexico, and California.

Host Studies.—The walnut is the preferred host of this insect, though infestations of minor importance have been observed in the peach under field conditions. Under laboratory conditions several larvae reached maturity in the tomato, and pupated. The late-maturing thick-husked varieties of walnut are most susceptible to attack. The nature of this very marked varietal susceptibility is apparently related to the hardness of the husk of the different varieties at the time of oviposition. The green husk tissue becomes harder as the walnut develops, reaching a peak of hardness usually during late June or early July, and then softening during late August and early September. Females are unable to puncture the husk for egg deposition at the peak of hardness; therefore oviposition takes place on the descending slope of the seasonal-husk-hardness curve. The husk of the so-called "resistant" varieties is generally not soft enough for egg deposition until several weeks prior to maturity of the nut and subsequent harvesting. Extensive data regarding husk hardness over a period of four years were obtained through the use of a modified Jolly balance.

Laboratory studies of possible hosts showed that females attempted oviposition in all fruits and tubers placed in cages with them. Eggs were deposited below the surface of the skin in tangerine, Mediterranean Sweet orange, apple, pear, quince, plum, prickly pear, potato, eggplant, and bell pepper. Larval maturity was not reached in any instance. Females vigorously attempted to insert eggs below the skin surface of the ripe Valencia orange and grapefruit. The texture of the lower skin tissue prevented them from making a cavity; however, the ovipositor was readily inserted into the tissue. Usually a single egg was placed into the small, shallow hole made by the ovipositor and other eggs were voided on the surface nearby. In all cases the eggs dried out before hatching.

Eggs were artificially placed in the pulp tissue of oranges, and larvae hatched and reached maturity within the fruit.

Injury and Economic Importance.—The principal type of injury results from the feeding of larvae within the green husk, thereby causing internal decay which permanently blackens the shell of the walnut. Such affected walnuts become "culls" with a resultant loss in value to the producer of approximately 50 per cent. The secondary type of injury is manifested by a reduction in quality of the kernels of infested nuts. The net loss in value varies from 0 to 25 per cent, according to seasonal conditions. Infested walnuts generally become "sticktights," resulting in increased harvesting costs. Other economic considerations are the costs incident to enforcement of regulatory measures for the prevention of artificial spread into uninfested areas.

Adult.—Methods were developed whereby various laboratory experiments involving thousands of adults were fairly satisfactorily conducted. An inverted battery jar, resting on plate glass and with wire-screen vent at the base, produced very favorable humidity conditions for longevity. Liquid food consisting of sucrose or honey was adsorbed in cotton and placed in the cages in one-half of a small petri dish.

Detailed records of adult emergence from the soil over a five-year period, involving over 37,000 flies, show that the seasonal peak varies considerably. Winter temperatures are apparently of prime importance in this connection. For the five-year period an average of 71 per cent of the flies emerged the year after pupation and are classed as annual-generation individuals; 29 per cent emerged the second year after pupation and are classed as biennial-generation individuals; while a few flies did not emerge until the third and fourth years after pupation and are classed as multi-annual-generation individuals. The daily rate of emergence within a single season is apparently influenced by daily mean temperature. High temperature is accompanied by high rate of emergence and low temperature by low rate of emergence. When the former condition exists, the major portion of those individuals that emerge during one day do so in the forenoon before the temperature reaches the daily peak.

Adults are attracted to a slight degree by the products of fermenting molasses. Outdoor laboratory tests, wherein over 100 organic chemicals were used in chemotropic studies, gave neutral results. Simple phototropic tests produced inconclusive data. Flies are apparently neutral in their reaction to anemotropic and thermotropic stimulation. They show strong negative geotropism immediately after emerging from puparia.

Honeydew, resulting from infestation by aphids, spores of yeast and fungi occurring naturally on the trees, and moisture, mainly in the form

of atmospheric dew, appear to constitute the food of the adult. Under field conditions the flies generally feed most actively in the morning hours after the sun rises and until the dew has completely evaporated. Laboratory studies demonstrated that the flies ingest very small, solid particles of matter.

Flies have been observed to travel on the wing from one tree to another. Other evidence regarding flight and dispersion is circumstantial: when food and oviposition conditions are favorable, the flies apparently remain localized on individual or closely adjacent trees; however, when either of these factors is adverse, migration is evidently stimulated.

Sucrose applied under uniform conditions to walnut foliage served to congregate a portion of the flies present, and thereby enabled a comparison of the relative density of the fly population on individual trees. The data show that the movement of flies on trees is closely related to sunshine, temperature, and humidity.

The average length of life of adults under laboratory conditions was approximately 40 days, and the most aged individuals lived 85 days. Under field conditions the average length of life is probably from 30 to 40 days. Without food, the greater portion of the flies died within 50 hours. When daily peak temperatures ranged from 95° to 100° F, the length of life was materially shortened; however, under optimum humidity conditions flies were not killed when the temperature remained at 114° F for several hours. High relative humidity is apparently a very important factor in longevity.

From preliminary nutritional studies, dealing with several proteins, carbohydrates, and minerals, the following indicative information was obtained.

Proteins: Apparently yeast decreased longevity and fecundity; glycocoll decreased longevity though it increased fecundity; and both urea and ammonia increased longevity and fecundity.

Carbohydrates: Apparently sucrose, levulose, dextrose, and honey were essential for longevity and fecundity; both honey and levulose reduced longevity slightly in comparison with sucrose, though fecundity was increased; dextrose reduced longevity in comparison with sucrose, without affecting fecundity; and dextrin had little or no food value.

Minerals: Apparently zinc and copper in the diet increased longevity and fecundity.

pH of media: Apparently no relation exists between food of pH from 3.8 to 9.5, and longevity and fecundity.

The most important male genital structures are illustrated. They are: the testes, seminal vesicles, vasa deferentia, accessory glands, ejaculatory duct, seminal pump, aedeagus, and claspers.

The most important female genital structures are illustrated. They are: the ovaries, oviducts, vagina, spermathecae, accessory glands, ovipositor sheath, and ovipositor.

Copulation first took place 6 or 8 days after the adults emerged from the soil. Under natural conditions it was most commonly observed during late afternoon, and usually immediately followed oviposition. The indications are that it was of frequent occurrence.

The first eggs in the ovaries were completely developed in from 10 to 20 days after the female emerged from the soil. This preoviposition period in a large number of individuals averaged 18 days under laboratory conditions.

It is estimated that under optimum field conditions females deposited from 200 to 400 eggs. Fecundity was greatly reduced in the laboratory and the maximum number of eggs deposited by one female was 84, in a total of 7 cavities. In oviposition, a cavity just below the surface of the husk is produced by the female, the ovipositor being used to lacerate the inner tissue. Eggs are deposited in batches of approximately 15 and usually all eggs within an individual cavity are deposited by one female at one insertion of the ovipositor. Eggs are deposited in healthy tissue only. Data for a four-year period show that 72 per cent of all cavities of eggs were located in the stem region of the husk, 24 per cent in the middle region, and 4 per cent in the calyx region. Females show a slight preference for the nuts in the middle and upper portions of the tree for oviposition, rather than in the lower portion.

Egg.—The average length of incubation period under field conditions was 120 hours. It was 72 hours under laboratory conditions. Egg mortality in the field was approximately 20 per cent and was mainly due to infertility and the work of natural enemies.

Larva.—Newly hatched larvae remained alive without food for 6 to 12 hours. The larvae consume only healthy tissue for food. They have gregarious tendencies in feeding. The average length of the instars under field conditions was: first instar, 9.7 days; second instar, 13.0 days; third instar, 14.1 days; and total development, 36.8 days. During the early portion of the season a number of larvae reached maturity in 18 or 20 days. Larval mortality within the walnut husk approximated 25 per cent.

When maturity was reached, the larvae issued from the husk and dropped to the soil, where they burrowed downward and later pupated. The greater percentage of daily emergence of larvae from the walnuts occurred between the hours of 5:30 and 8:00 a.m., which fact indicates a relation with temperature. The larvae show marked positive geotropism, and apparently react negatively to light. The depth that larvae

penetrated the soil to pupate varied from ½ to 7 inches, according to the soil type, degree of moisture present, and the existing state of cultivation.

Limited laboratory studies regarding the effect of temperature on larvae showed that at 30° F for 35 hours the mortality was fairly high; however, when removed and placed at 72° F, pupation was materially stimulated by the exposure to low temperature. An exposure of ½ hour at 115° F proved fatal to a high percentage, while exposure for ¾ hour resulted in total mortality.

Pupa.—The puparium was completely formed within 24 hours after the larva entered the soil. An additional larval molt occurred within 36 hours after the formation of the puparium. The true pupal stage was reached within 145 to 175 hours after the larva entered the soil. Normal summer temperatures of the soil in total sunlight proved fatal to pupae located in the upper portion of the soil; however, most of the pupae were buried to a depth of from 3 to 8 inches by the usual cultivation practices. Pupal mortality was variable, though considerable, in all instances recorded.

Data obtained regarding dormancy indicate that a fairly definite amount of heat units supplied by a range of fluctuating temperatures are important in the termination of this condition. Tests of various chemicals showed that both potassium thiocyanate and thiourea apparently exerted a slight effect upon the termination of dormancy.

Seasonal History.—The data show that accumulated soil-temperature conditions during dormancy and host resistance exert a profound effect upon seasonal activity of the fly. The relation of winter temperatures to time of seasonal emergence has already been pointed out.

Oviposition data show that the peak of egg laying each year for the five-year period was reached between August 29 and September 5, despite wide variation in the seasonal median of adult emergence. Evidently hardness of the green husk is the most important physical factor relating to time of oviposition as well as to varietal susceptibility.

The earliness of walnut harvest influences larval emergence from the husks. Under average conditions approximately 75 per cent have issued at the time of harvest; however, in 1932 harvest was about 20 days earlier than normal and only 24 per cent had emerged at that time.

Natural Enemies.—The walnut husk fly is remarkably free from important natural enemies. Several species of parasitic fungi have been cultured from dead adults. The mite, *Pediculoides ventricosus* New., and the anthocorid, *Triphleps insidiosus* (Say), prey upon the eggs. Several other species of common predators, including spiders, a reduviid, a chrysopid, and ants, feed upon the larvae, pupae, or adults. Two

general feeding parasites of dipterous pupae, the chalcid, *Spalangia rugosicollis* Ash., and the proctotrupid, *Galesus* sp. near *atricornis* Ash., have been reared from *Rhagoletis completa* at Manhattan, Kansas. The opiine larval parasites, *Opius humilis* Silv. and *Diachasma tryoni* Cam., have been introduced from Hawaii, and the former was recovered in the field in 1932.

Scavenger Species Inhabiting Decaying Walnut Husks.—More than 30 species of scavenger insects, mainly Diptera, have been reared from decaying walnut husks. The flies, *Euxesta putricola* Cole, *Lonchaea occidentalis* Mall., *Muscina assimilis* Fall, *Fannia canicularis* (Linn.), and *Drosophila* spp., are most commonly observed. All scavengers are of negligible economic importance.

Laboratory Toxicological Investigations.—A satisfactory method was devised whereby a comparison of the relative speeds of toxic action of various materials upon adult flies was possible. Tests of this nature were conducted during 1929, 1930, 1931, and 1932. The results of these tests are graphically presented. Of the arsenicals tested, basic lead arsenate was consistently slowest in speed of lethal action, though aside from magnesium arsenate it was the only one of the group that did not cause foliage injury. With respect to magnesium arsenate, one season's observations indicate that it is safe to use on walnut foliage; however, its lethal action is apparently not appreciably more rapid than that of basic lead arsenate. Of the fluorine compounds tested, synthetic cryolite (sodium fluoaluminate), barium fluosilicate, and potassium fluoaluminate are the most promising from the point of view of toxicity to the insect and tree tolerance. The copper compounds tested offer promise with respect to lethal action, though they are deleterious to walnut foliage. The nicotine compounds and combinations tested are effective as stomach poisons on the flies, though they do not appear to be as practicable in the field as the several fluorines mentioned. Of the inert diluents, either diatomaceous earth or talc is satisfactory. Hydrated lime employed as a diluent with synthetic cryolite materially retarded lethal action. All diluent materials tested, whether chemically active or inert, were lethal to the flies. The nature of their action is unknown; however, hypotheses are suggested for the mode of action of several of these materials. The incorporation of either mineral oil, vegetable oil, or fish oil at a concentration of 5 per cent for adhesive purposes, did not retard lethal action of the insecticide dust mixture. However, at a concentration of 12 per cent, both mineral oil and fish oil apparently inhibited the speed of toxic action.

Field Investigations.—The field investigations dealt mainly with plot experiments to determine the efficacy of various materials, concentra-

tions, and methods and time of application. Wherever possible, plots were isolated from each other and from adjoining properties to preclude the undue influence of uncontrollable variable factors. The application in all plots was very thorough and the infestation data were analyzed with the aid of simple statistical methods. In many instances erratic and inconclusive data were obtained. A summary of the more important results of the field experiments follows:

Available information indicates that control of the fly by altering the environment through manipulation of soil moisture in an effort to inhibit oviposition activities is impractical.

The application of basic lead arsenate does not afford a satisfactory means of controlling the fly though it is generally most effective when applied two or three times as a spray, and when thorough coverage of the trees is obtained. Synthetic cryolite and barium fluosilicate applied as a spray or dust are satisfactory insecticides for controlling this insect.

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